IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	Cristobal Guillermo dos Remedios	**Examiner:**	Changhwa J Cheu
Serial No:	09/778,259	**Art Unit:**	1646
Filed:	July 2, 2001	**Docket:**	13388
For:	BIOMOLECULAR TOXICITY ASSAY		

Confirmation No.: 4496

Commissioner for Patents
United States Patent and Trademark Office
Alexandria, Virginia 22313-1450

DECLARATION OF PROFESSOR CRIS DOS REMEDIOS UNDER 37 C.F.R. §1.132

I, Cris dos Remedios, hereby declare:

1. I am Professor of Anatomy and Biophysics at The University of Sydney, and a named inventor of the above-identified patent application. A copy of my curriculum vitae is attached as Exhibit I.

2. I have reviewed the relevant documents from the prosecution of the Application, including the Office Action dated September 11, 2007 and the art cited therein. I understand that the claims have been rejected as allegedly obvious in light of Liu *et al.* (*J. Inorganic Biochemistry*, 1998, Vol. 71, pages 1-6) in light of Pisanti *et al.* (*Marine Pollution Bull.*, 1998, Vol. 19, pages 328-333), and further in view of Gold *et al.* (US Patent No. 6,242,246). I have been asked to comment on the relevance of the cited references to the methods claimed in the above-identified patent application ("the present application").

3. I observe that the claims pending in the present application are directed to methods of detecting the presence of micromaloar concentrations of a toxicant

in an aquatic, terrestrial, gaseous or industrial sample, wherein the toxicant is a heavy metal ion. The method involves the steps of contacting a sample with a nucleic acid molecule intercalated with a fluorescent dye; and screening for dissociation or inhibition of binding between the nucleic acid molecule and the dye, wherein the dissociation or inhibition of binding is indicative of the presence of a toxicant.

4. The primary reference to Liu *et al.* dislcoses the fluorescence quenching of DNA bound to ethidium bromide by two specific Cu (II) macrocyclic complexes in order to estimate binding constants between the complexes and the DNA. The Cu (II) macrocyclic complexes studied by Liu *et al.* were prepared and purified by the literature methods as described in Reference No. 19 cited by Liu *et al.* (Truex and Holm) (a copy of which is attached as Exhibit II) and reference No. 20 (Lui). Exhibit II teaches that (see for example, page 4530, first column, lines 4-8) naturally occurring macrocycles are usually of the 6-6-6-6 or 6-6-6-5 type. In contrast, the Cu (II) complexes studied by Liu *et al.* contain alternating 6- and 5-membered rings *i.e.* are of the 6-5-6-5 type. Accordingly, Liu *et al.* disclose fluorescence quenching of DNA bound ethidium bromide by two specific **non-natural** Cu (II) complexes which were prepared for the purpose of estimating binding constants.

5. To my knowledge, the **non-natural** Cu (II) complexes disclosed by Liu *et al.* would not be the target of the claimed methods which are designed to detect toxic levels of **heavy metal ions** in, for example, environmental samples. Further to my knowledge, the **non-natural** Cu (II) complexes disclosed by Liu *et al.* normally would not be expected to exist in an environmental sample.

6. In fact, the non-natural Cu (II) complexes used by Liu *et al.* were selected as these complexes have a high affinity for DNA. Accordingly, the method of Liu *et al.* is very sensitive and will detect very low, non-toxic levels of metal complexes. It is submitted that the high sensitivity of the Liu *et al* method is afforded by the high affinity of the macrocyclic metal complexes for DNA resulting in a greater ability of the complexes to inhibit the DNA-dye interaction.

7. In contrast, the present invention is directed to detecting a toxicant that is a heavy metal ion which is present in, for example, an environmental sample, at

toxic levels. In contrast to Liu *et al.* the claimed method, which does not employ any macrocyclic compound is more discriminating so that non-toxic levels of metal ions are not detected. Indeed, the methods as taught by Liu *et al.* would not be able to be used in the methods of the present invention as the Liu *et al.* assay would be unable to discriminate between toxic and non-toxic levels of heavy metal ions and therefore would result in false positives being generated.

8. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Professor Cris dos Remedios

December 18, 2007
Date

Cristobal G. dos Remedios. Professor of Anatomy and Biophysics, Bosch Institute, Department of Anatomy & Histology, The University of Sydney 2000-.
Nationality: Australian; born in Kobe Japan, 19-12-1940; married 42 years, four sons.

Scientific Societies & Bodies:

- Former President of the Australian Society for Biophysics.
- Chair, National Committee for Biophysics, Australian Academy of Science, 1994-2001.
- Member of the Council (1997-2003), Executive (2004-2005) of the US Biophysical Society. I served on its International and Publications Committees.
- Vice-President of the International Union for Pure & Applied Biophysics (2004-06).
- Secretary General (elect) IUPAB (2008-2014).
- Director, Institute for Biomedical Research, University of Sydney (1998-2000).
- Associate Dean, Faculty of Medicine, University of Sydney (1999-2002).
- International Council for Science (ICSU) member, *ad hoc* committee (2002-)
- Member of the Scientific Advisor Board, Medsaic Pty Ltd. (2005-07).
- Director, HazardScreen Pty Ltd (2001-present).

Invited lectures: (a selection, 2002-07) IUPAB Congress, New Delhi, Buenos Aires & Montpellier; London Muscle Conference; Mayo Clinic Rochester; Univ. Northern Texas, Dallas; Harvard Medical School, Brigham & Women's Hosp. Boston; ICSU Conf. on Ethics in Science, Como, Italy; British Society for Proteomic Research, Cambridge; Centre for Proteomics Research, University College Dublin; CNRS, INSERM U554 Nantes, France; Molecular Pharmacology, Univ. Miami; Physiology, Univ. Maryland, Baltimore; Distinguished Lecturer in Cardiovascular Sciences, UCLA; Biochemistry & Biophysics, Univ. California San Francisco; Johns Hopkins Univ. Regional Biosecurity Conference, Singapore; Molecular Biology & Biochemistry, Univ. California Los Angeles.
I convened a meeting on Molecular Defects in Human Heart Failure, Imperial Coll. London.

Editorial Boards:

(1) *Biophysical Journal*, Biophysical Society Press.
(2) *Proteomics*, Wiley and Sons.
(3) *Proteomics Clinical Application*, Wiley and Sons.
(4) *Biophysics* (Japanese Biophysical Society).

Community Service:

NSW section of the Churchill Foundation Fellowship Committee 2005-08.

Inventor: I hold Australian and International patents on two separate inventions:

(1) a microarray of antibodies (Medsaic P/L) that uses white blood cells isolated from 5 mL of blood to diagnose and detect changes in malignancy in human cancers. (leukemias, melanoma, breast cancer) with co-inventor Prof. R.I. Christopherson;
(2) a bioassay used to monitor toxic molecules (heavy metal ions, organic molecules) in water samples (European patent granted) with co-inventors AR Cooke and M Kekic.

Name: Cristobal Guillermo dos Remedios

Qualifications:
B.Sc. (1965, Univ. Sydney); Ph.D. (1969, Univ. Sydney), D.Sc. (1995, Univ. Sydney)

Current Appointments:
Professor of Anatomy & Biophysics (appointed 2001)
Department of Anatomy & Histology,
University of Sydney (appointed 1974, Assis/Prof 1980-2000)
Director, Muscle Research Unit, Department of Anatomy & Histology, University of Sydney (1974-present)

Past Appointments:
US Biophysical Society, Member of Council (1998-2003), elected to Executive, (2003-2004). Member of International Committee (2003-2006)
Director, Institute for Biomedical Research, Univ Sydney (1997-1999)
Associate Dean, Univ Sydney (1999-2001)
Associate Professor of Anatomy, Univ Sydney, 1980-2000
Senior Lecturer, Univ of Sydney, 1974-1979
Senior Lecturer, Univ of New South Wales 1972-1974
Career Investigator Fellow, American heart Association, 1969-1972.

National and International Professional Societies
IUPAB: Vice-President (2002-2004), and a member of Council (1996-present)
US Biophysical Society, 1972-present Member of Council 1999-2002, Member of National Executive 2002- 2004, Editorial Committee 2006-
Australian Society for Biophysics, 1978-present, President 1995-1997
National Committee for Biophysics of the Australian Academy of Science, member 1989-91, Chair 1995-2001.
Australian Society for Medical Research, 1987-present
Australian Physiological and Pharmacological Society, 1983-present
International Society for Heart Research, 1998-present
Australian Science Communicators, 2000-present
Anatomical Society of Australia and New Zealand, 1972-present

Awards, Honours
Career Investigator Fellow, American Heart Association, 1970-1972
Louis N Katz Prize for young investigators, American Heart Association, 1971
Invited symposia/lectures (recent): International Union for Pure and Applied Biophysics, Delhi 2004, Buenos Aires 2004; British Proteomics Society 2006; Australian Society for Biophysics 2005; Japanese Biophysical Society 2006; Aust & NZ Society for Clinical Cardiology, 2006; University of Maryland 2006; University of Miami School of Pharmacology 2006.
Distinguished Cardiovascular Lectureship, University of California Los Angeles 2006.
Visiting Professor, Mayo School of Medicine;

Membership of Editorial Boards

Electrophoresis	Associate Editor of 1996-2003
Proteomics	Editorial Board, 2003-present

Proteomics Clinical Applications	Editorial Board, 2007-present
European Biophysics Journal	Editorial Board, 2003
Biophysics Editorial Board, Biophysical Society of Japan, 2005- present	
Biophysical Journal	Editorial Board, 2006-2008

Publications

Published papers (#1 paper was cited as one of the 50 important papers in the history of muscle research and myosin motility. *Journal of Muscle Research and Cell Motility* 25: 475-479).

1. dos Remedios CG, Millikan RGC, and Morales MF. (1972) Polarization of tryptophan fluorescence from single striated muscle fibres. A molecular probe of contractile state. *The Journal of General Physiology* 59: 103-120.
2. dos Remedios CG, Yount RG, and Morales MF. (1972) Individual states in the cycle of muscle contraction. *Proceedings of the National Academy of Sciences of the United States of America* 69: 2542-2546.
3. Botts J, Cooke R, dos Remedios CG, Duke J, Mendelson R, Morales MF, Tokiwa T, Viniegra G, and Yount R. (1973) Does a myosin cross-bridge progress arm-over-arm along the actin filaments? *Cold Spring Harbor Symposium on Quantitative Biology* 37: 195-200.
4. McGrath PA, and dos Remedios CG. (1974) The dependence of rigor tension on sarcomere length in vertebrate muscle. *Experientia* 30: 1036-1038.
5. dos Remedios CG. (1976) Lanthanide ions and skeletal muscle sarcoplasmic reticulum. *Journal of Biochemistry* 81: 703-708.
6. dos Remedios CG. (1977) Ionic radius selectivity of skeletal muscle membranes. *Nature* 270: 750-751.
7. dos Remedios CG, and Barden JA. (1977) Effects of Gd(III) on G-actin: Inhibition of polymerization of G-actin and activation of myosin ATPase activity by Gd-G-actin. *Biochemical and Biophysical Research Communications* 77: 1339-1346.
8. Hambly BD, and dos Remedios CG. (1977) Responses of skeletal muscle fibres to lanthanide ion. Dependence of the twitch response on ionic radii. *Experientia* 33: 1042-1043.
9. dos Remedios CG, and Gilmour D. (1978) Is there a third type of filament in striated muscle? *Journal of Biochemistry* 84: 235-238.
10. dos Remedios CG, and Dickens MJ. (1978) Actin microcrystals and tubes formed in the presence of gadolinium ions. *Nature* 276: 731-733.
11. Barden JA, and dos Remedios CG. (1978) Evidence for the non-filamentous aggregation of actin induced by lanthanide ions. *Biochimica et Biophysica Acta* 537: 417-427.
12. Barden JA, and dos Remedios CG. (1979) Binding stoichiometry of gadolinium to actin: Its effect on the actin-bound divalent cation. *Biochemical and Biophysical Research Communications* 86: 529-535.
13. Barden JA, and dos Remedios CG. (1980) Structural similarities and differences between crystalline actin aggregates formed in the presence of the lanthanide ions. *Micron* 11: 285-286.
14. Barden JA, and dos Remedios CG. (1980) Crystalline actin tube. I Is the conformation of the lanthanide-induced actin tube monomer more like F-actin than G-actin? *Biochimica et Biophysica Acta* 624: 163-173.

15. dos Remedios CG, Barden JA, and Valois AA. (1980) Crystalline actin tubes. II The effect of various lanthanide ions on actin tube assembly. *Biochimica et Biophysica Acta* 624: 174-186.
16. Cartmill JA, and dos Remedios CG. (1980) Ionic radius specificity of cardiac muscle membranes. *Journal of Molecular and Cellular Cardiology* 12: 219-223.
17. Barden JA, Cooke R, Wright PE, and dos Remedios CG. (1980) Proton nuclear magnetic resonance and electron paramagnetic resonance studies on skeletal muscle actin indicate that the metal and nucleotide binding sites are separate. *Biochemistry* 19: 5912-5916.
18. Barden JA, and dos Remedios CG. (1981) Crystalline actin tubes. III The interaction of scandium and yttrium with skeletal muscle actin. *Biochimica et Biophysica Acta* 672: 25-32.
19. Barden JA, Hambly, BD, and dos Remedios CG. (1981) A comparison of the binding of myosin and spectrin oligomers to actin. *Biochemistry International* 2: 411-419.
20. dos Remedios CG. (1981) Lanthanide ion probes of calcium-binding sites on cellular membranes. *Cell Calcium* 2: 29-51.
21. Finlayson PJ, and dos Remedios CG. (1981) Differences between cardiac and skeletal muscle actins. *Journal of Cellular and Molecular Cardiology* 13: 1081-1086.
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24. Barden JA, Grant NJ, and dos Remedios CG. (1982) Identification of the nucleus of actin polymerization. *Biochemistry International* 5: 685-692.
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41. Barden,JA, and dos Remedios CG. (1987) Fluorescence resonance energy transfer between sites in G-actin. The spatial relationship between Cys-10, Tyr-69, Cys-374, the high affinity metal and the nucleotide. *European Journal of Biochemistry* 168: 103-109.
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58. Miki M, O'Donoghue SI, and dos Remedios CG. (1992) Structure of actin observed by fluorescence resonance energy transfer spectroscopy. *Journal of Muscle Research and Cell Motility* 13: 132-145.
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64. Moens P, Yee D, and dos Remedios CG. (1994) Determination of the radial coordinate of Cys-374 in F-actin using fluorescence resonance energy transfer

spectroscopy: Effect of phalloidin on polymer assembly. *Biochemistry* 33: 13102-13108.

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79. dos Remedios CG, and Moens PDJ. (1995) Fluorescence resonance energy transfer spectroscopy is a reliable "ruler" for measuring structural changes in proteins - Dispelling the problem of the unknown orientation factor. *Journal of. Structural Biology* 115: 175-185.
80. dos Remedios CG, and Moens PDJ. (1995) Actin and the actomyosin interface: A review. *Biochimica et Biophysica Acta* 1228: 99-124.
81. Yao M, Keogh A, Spratt P, dos Remedios CG, and Kiessling PC. (1996) Elevated DNase I levels in human idiopathic dilated cardiomyopathy: An indicator of apoptosis? *Journal of Molecular and Cellular Cardiology* 28: 95-101.
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89. Coumans JVF, Yeoh T, Seeto RK, Keogh A., Brennan K, Gunning P, Hardeman E, and dos Remedios CG. (1997) Actin-binding proteins in mouse C2 myoblasts and myotubes: A combination of affinity chromatography and two-dimensional gel electrophoresis. *Journal of Molecular and Cellular Cardiology* 29: 895-905.
90. dos Remedios CG, and Simpson R. (1998) Bright sparks in Australian 2-DE: Report on the fourth annual conference of the Australian Electrophoresis Society. *Electrophoresis* 19: 807-808.
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92. Coumans JVF, and dos Remedios CG. (1998) Actin-binding proteins in mouse C2 myoblasts and myotubes: A combination of affinity chromatography and two-dimensional gel electrophoresis. *Electrophoresis* 19: 826-833.

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anisms, unless a radical-forming step is reversible. A separate rate term may exist, however, of the form k-$[Cu(SO_4)_n^{2-n}][S(IV)]$. A similar Cu(II) term can contribute significantly to the slower sulfur(IV) reduction of chloroaquoiron(III) complexes, in 1 M chloride media,[36] but was not observed in the sulfur(IV) reduction of sulfatoaquoiron(III) complexes in sulfate media.[6]

Oxidation of sulfur(IV) by a pathway second order in sulfur(IV) is unusual. The previous observation of a second-order path, in the oxidation by chromium(VI), probably involves reaction between a sulfitochromate(VI) ester and a second sulfur(IV) species.[37] Mechanism 7 is similar to that proposed for the chromium system,[37] except for the structure of the intermediate; ester formation by FeL_3^{3+} does not appear possible. A mechanism analogous to 6 could not account for the exact form of the rate law in the chromium system,[37] rate $= k[Cr(VI)][S(IV)]^2[H^+]/(1 + a[S(IV)])$, owing to the small value[35] of Q_{6a}.

Limiting mechanisms 6 and 7, representing approach to the transition state by two sulfur atoms, either in a single species (6), or separately (7), cannot be distinguished by the observations made in this study. The positive entropy of activation appears consistent with either mechanism. If mechanism 6 is correct, then comparatively great reactivity of $S_2O_5^{2-}$ in this system is implied; second-order terms could perhaps be found in other systems, including anion oxidants such as $IrCl_6^{2-}$, for which ion pairs or specific sulfur–ligand interactions are unlikely.

If mechanism 7 is correct, then one can question whether the species $(FeL_3 \cdot S)^{VII}$ is only an ion pair or if a more specific interaction between inner- and outer-sphere ligands exists. An experimental approach to this question would include attempts to detect the complex, or other $ML_3 \cdot S$ complexes, and studies of reaction with other metal ion oxidants, especially highly charged ones. It has been suggested[38] that anion catalysis of the $Fe(H_2O)_5^{2+}$–FeL_3^{3+} reaction may involve specific interaction between a bridging anion and the phenanthroline ligand.

The unexpected results of this work are the suggestions that $S_2O_5^{2-}$, potentially a two- to four-electron reducing agent, may react rapidly in a one-electron step and an alternate possibility that decomposition of $(FeL_3 \cdot S)^{VII}$ to FeL_3^{2+} and S(V) can be accelerated by S(IV).

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Synthesis and Properties of Tetraaza[14]tetraene and Tetraaza[14]hexaene Macrocyclic Complexes

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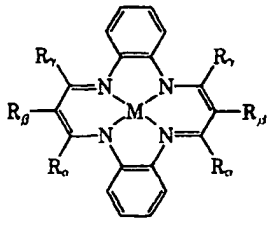
Abstract: An efficient nontemplate synthesis of the new macrocycle 5,7,12,14-tetramethyl-1,4,8,11-tetraazacyclotetradeca-4,6,11,13-tetraene (H_2(MeHMe(en)$_2$), **11**) is described. This compound reacts with metal(II) acetates or with other metal(II) salts under conditions of nonaqueous chelation to afford the macrocyclic 12-π complexes M(MeHMe(en)$_2$) (**13**, M = Fe(II), Co(II), Ni(II), Cu(II), Zn(II)), whose electronic properties are consistent with planar coordination. Treatment of the Ni(II) and Cu(II) complexes with 3 equiv of trityl tetrafluoroborate in acetonitrile results in oxidative dehydrogenation, yielding the 15-π cations [M(MeHMe-2,9-diene)]$^+$ (**15**) which were isolated as their tetrafluoroborate salts. Conductivity, spectrophotometric, and epr results indicate that the nickel complex exists in a paramagnetic monomer $\rightleftarrows$ diamagnetic dimer equilibrium in solution. The nickel and copper cations are reduced by sodium borohydride in ethanol to the neutral 16-π complexes M(MeHMe-2,9-diene) (**14**). Voltammetric studies of these complexes in acetonitrile have established the existence of a three-membered electron-transfer series **14** (16-π) $\rightleftharpoons$ **15** (15-π) $\rightleftharpoons$ **16** (14-π). Members of the series are interconverted by reversible one-electron redox processes considered to alter the ligand oxidation level such that the terminal oxidized member, [M(MeHMe-2,8,10-triene)]$^{2+}$ (**16**), contains a ($4n + 2$)-stabilized ring system. 16-π Ni(II) complexes **26** derived from several recently reported dihydrooctaaza[14]annulene macrocycles have been prepared. These and the 16-π Ni(II) complexes **1** were found to undergo two-electron oxidations, presumably yielding the 14-π species **27** and **28**, respectively.

Attempts to relate electronic properties and reactivities of synthetic macrocyclic complexes to those of naturally occurring macrocycles, such as porphyrins and corrins, continue to promote considerable interest in their design and preparation. Work in this area has resulted in substantial progress in the development of cyclization reactions, usually involving metal ions, which have led to a variety of new macrocycles.[1] In addition, development of systematic transformations, particularly hydrogenation and dehydrogenation reac-

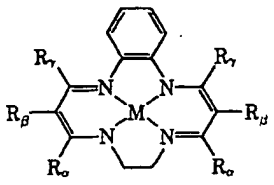
(1) (a) D. H. Busch, *Helv. Chim. Acta, Fasciculus Extraordinarius Alfred Werner*, 174 (1967); (b) D. H. Busch, K. Farmery, V. Goedken, V. Katovic, A. C. Melnyk, C. R. Sperati, and N. Tokel, *Advan. Chem. Ser.*, No. 100, 44 (1971); (c) J. P. Candlin, K. A. Taylor, and D. T. Thompson, "Reactions of Transition Metal Complexes," Elsevier, Amsterdam, 1968, pp 83–99; (d) L. F. Lindoy and D. H. Busch, *Prep. Inorg. React.*, 6, 1 (1971).

tions, has allowed preparation of species with lesser or greater degrees of unsaturation than the precursor macrocyclic complex.

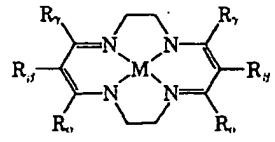
In contrast to the natural macrocycles, which usually contain four conjugated six-membered rings or three six- and one five-membered ring (6-6-6-6 and 6-6-6-5 types, respectively), the majority of synthetic macrocycles are of the 6-5-6-5 type.[2] Their complexes may be organized in terms of the degree of unsaturation of the ligand structure internal to the chelate rings. Known examples range from the completely conjugated 16-π electron system **1**[3-6] to the completely saturated system **6**[7] (and *C*-methyl derivatives thereof[8]) and include the 14-π[9] (**2**), 12-π[9-11] (**3**), 8-π[8,12] (**4**), and 4-π[8,13,14] (**5**) systems. Cis and trans 6-π and 2-π com-



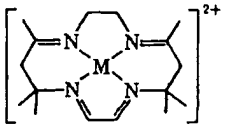
1, $M(R_\gamma R_\beta R_\alpha\text{-(phen)}_2)$



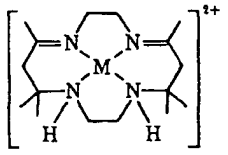
2



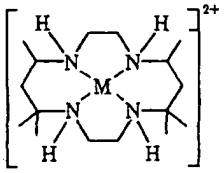
3, $M(R_\gamma R_\beta R_\alpha\text{-(en)}_2)$



4 (cis, trans)

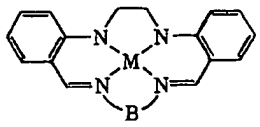


5 (cis, trans)



6

plexes derived from **5** have also been reported.[8,12a] In addition, the neutral complexes **7** with B = phen and en, which have been prepared recently,[15] may be recognized as 14-π and 12-π cases, respectively.



7

In the course of our examination of electronic properties and chemical reactivities of divalent metal ions in planar or tetragonal N_4 environments, we have devised a nontemplate synthesis of the basic macrocycle 5,7,12,14-tetramethyl-1,4,8,11-tetraazacyclotetradeca-4,6,11,13-tetraene, H_2(MeHMe(en)$_2$), and its 12-π metal(II) complexes M(MeHMe(en)$_2$), M = Fe(II)–Zn(II).

This report elaborates our recent account[16] of this work and also describes a new synthetic approach to the dehydrogenation of coordinated ligands which has resulted in the preparation of Ni(II) and Cu(II) complexes of the new 16-π macrocycle ligand 5,7,12,14-tetramethyl-1,4,8,11-tetraazacyclotetradeca-2,4,6,9,11,13-hexaene. Spectral and limited magnetic properties are presented for the above complexes and several members of another new class of 16-π M(II)–N_4 macrocyclic complexes derived from one type of recently reported dihydro-1,2,4,5,8,9,11,12-octaaza[14]annulenes.[17] Electrochemical and synthetic studies have been carried out on a series of 16-π complexes in order to determine if they can be oxidized to the 14-π level. Species of the latter type would presumably contain a $(4n + 2)$-stabilized macrocycle representing aza analogs of the carbocycle [14]annulene.[18]

Experimental Section

Preparation of Compounds. Ni(HHH(phen)$_2$), Ni(MeHH(phen)$_2$), and Ni(Me, COMe, H(phen)$_2$) were prepared by published methods.[4,6] Analytical data for new compounds are given in Table I. Spectral and other properties are summarized in Tables II–V. Structural formulas are set out in Scheme I and in the text.

5,7,12,14-Tetramethyl-1,4,8,11-tetraazacyclotetradeca-4,6,11,12-tetraene, H_2(MeHMe(en)$_2$) (11) and Its Metal(II) Complexes, M(MeHMe(en)$_2$) (13). H_2(MeHMe(en)$_2$). To a solution of 30.0 g (0.30 mol) of 4-aminopent-3-en-2-one[19] in 200 ml of dry dichloromethane was added 57.0 g (0.30 mol) of triethyloxonium tetrafluoroborate dissolved in 150 ml of dry dichloromethane. The solution was stirred at room temperature under an atmosphere of dry nitrogen for 30 min. Ethylenediamine (9.0 g, 0.15 mol), freshly distilled from sodium hydroxide, was added dropwise with stirring over a 30-min period. The solution was stirred for an additional 3 hr at room temperature during which time a white solid formed. Dichloromethane was removed under reduced pressure and replaced with 400 ml of absolute methanol. To this solution was added 16.2 g (0.30 mol) of sodium methoxide in 150 ml of methanol and thereafter an additional 9.0 g (0.15 mol) of ethylenediamine was immediately introduced. The reaction was allowed to proceed for 4 hr with stirring during which time ammonia was evolved. Removal of the methanol under reduced pressure, followed by extraction of the residue with hot absolute ethanol, yielded a first crop of cream-colored platelets from the cooled extract. A second crop was obtained by extraction of the residue with chloroform and removal of this solvent under reduced pressure. The two crops were combined and recrystallized from *ca.* 1800 ml of absolute ethanol to afford 13.0–13.2 g (35–35.5%) of pure product as cream-colored platelets. The product is best stored in a dry nitrogen atmosphere. Molecular weight: calcd, 248; found, 244 (osmometry, toluene solution). The mass spectrum revealed an intense parent ion peak at *m/e* 248.

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Table I. Characterization Data for Macrocyclic Ligand and Complexes

Compound	Mp,[a] °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
H_2(MeHMe(en)$_2$)	226–228	67.70	9.74	22.56	67.83	9.70	22.65
[H_4(MeHMe(en)](BF$_4$)$_2$	175–180	36.22	6.08	14.08	36.34	6.05	13.68
Fe(MeHMe(en)$_2$)	340–343	55.64	7.34	18.54	55.67	7.07	18.33
Co(MeHMe(en)$_2$)	>360	55.08	7.26	18.35	54.63	7.06	18.24
Ni(MeHMe(en)$_2$)	331–334	55.12	7.27	18.36	55.15	7.15	18.32
[Ni(H(MeHMe(en)$_2$))](BF$_4$)	247–251	42.69	6.04	14.22	43.15	6.03	14.31
[Ni(MeHMe-2,9-diene)](BF$_4$)[b]	262–263	43.24	4.93	14.41	43.64	4.80	14.54
Ni(MeHMe-2,9-diene)	310–312 dec	55.86	6.03	18.61	55.89	5.96	18.58
Ni(Me$_2$(ch)oaa)	273–275	49.90	5.76	29.10	50.08	5.93	29.29
Ni(Et$_2$(ch)oaa)	257–259	52.33	6.34	27.12	52.22	6.55	27.14
Cu(MeHMe(en)$_2$)	298–299	54.26	7.16	18.08	54.29	7.09	18.18
[Cu(MeHMe-2,9-diene)](BF$_4$)	255–256	42.81	4.62	14.26	43.25	4.83	14.41
Cu(MeHMe-2,9-diene)	320–322	54.98	5.93	18.31	55.08	6.10	18.31
Zn(MeHMe(en)$_2$)	266–268	53.94	7.11	17.97	54.23	6.76	17.96

[a] Sealed tube, uncorrected. [b] Calculated for [Ni(MeHMe-1-ene)](BF$_4$): C, 43.02; H, 5.41; N, 14.33.

Table II. Pmr Data for Macrocycles and Complexes

Compound	Solvent	Chemical shifts, ppm
H_2(MeHMe(en)$_2$)	$CDCl_3$	−1.95 (Me), −3.51 (CH_2), −4.58 (CH), −11.6[a] (NH)
[H_4(MeHMe(en))](BF$_4$)$_2$[b]	py-d_5	−2.36[c] (Me), −3.83[d] (CH_2), −5.49 (CH), −8.80[a] (NH)
Ni(MeHMe(en)$_2$)	$CDCl_3$	−1.90 (Me), −3.12 (CH_2), −4.58 (CH)
[Ni(H(MeHMe(en)$_2$))](BF$_4$)	DMSO-d_6	−2.01 (Me), −2.14 (Me), −3.40[d] (CH_2), −4.19 (CH), −4.92 (NH)
Zn(MeHMe(en)$_2$)[e]	$CHCl_3$	−1.97 (Me), −3.52 (CH_2), −4.47 (CH)
H_2(Me$_2$(ch)oaa)	$CDCl_3$	−1.65[d] (β-CH_2), −2.10 (Me), −2.32[d] and −2.70[d] (α-CH_2), −17.4 (NH)
H_2(Et$_2$(ch)oaa)	$CDCl_3$	−1.18[f] (Me), −1.65[d] (β-CH_2), −2.30[d] and −2.72[d] (α-CH_2), −2.50[f] (CH_2), −17.2 (NH)
Ni(Me$_2$(ch)oaa)	$CDCl_3$	−1.67[d] (β-CH_2), −2.09 (Me), −2.50[d] and −2.85[d] (α-CH_2)
Ni(Et$_2$(ch)oaa)	$CDCl_3$	−1.13[f] (Me), −1.70[d] (β-CH_2), −2.48[d] and −2.88[d] (α-CH_2), −2.56[f] (CH_2)

[a] Center of broad peak. [b] A second small peak in the (CH) region at −5.21 ppm (relative intensity ~10%) indicates the presence of a second conformation in solution or an impurity of similar structure. [c] Center of two overlapping singlets. [d] Center of a complex multiplet. [e] Spectrum taken in $CHCl_3$ because of loss of (CH) signal due to exchange in $CDCl_3$. [f] Centers of triplet and quartet of ethyl groups, J_{HH} = 7.5 Hz.

Table III. Magnetic and Electronic Spectral Data

Compound	μ_{eff} (BM)[a]	λ_{max}, cm^{-1} (ϵ)[b]
H_2(MeHMe(en)$_2$)[c]		30,200 (sh, 5800), 32,600 (28,700), 33,100 (sh, 25,200)
Zn(MeHMe(en)$_2$)[c]	dia	~18,500 (sh, 70), ~25,100 (sh, 1400), ~28,300 (sh, 10,100), 30,300 (17,000)
Fe(MeHMe(en)$_2$)[d]	3.95	7690 (87), ~10,100 (sh, 140)
Co(MeHMe(en)$_2$)[d]	2.06	10,870 (45), ~17,000 (sh, ~280), ~19,600 (sh, 790), ~21,500 (sh, 1290), ~23,200 (sh, ~1700), 25,800 (5230), ~27,800 (sh, 4200), 32,200 (7700), 37,500 (9130)
Ni(MeHMe(en)$_2$)$_2$[c,g]	dia	17,900 (227), 21,500 (sh, 1200), 23,600 (sh, 3700), 24,950 (6090), ~30,400 (sh, ~4400), 34,500 (15,200), ~38,900 (sh, ~10,300)
[Ni(H(MeHMe(en)$_2$))](BF$_4$)[e]	dia	18,700 (186), 21,600 (sh, 140), ~24,400 (sh, ~550), 25,400 (sh, 1250), 28,100 (9930), 29,100 (sh, 8300), 36,300 (5060), 38,600 (6080)
[Ni(MeHMe-2,9-diene)](BF$_4$)[f,h]	dia	7500 (565), 11,100 (2320), ~13,800 (sh, 1900), ~16,600 (sh, 1400), 18,800 (1740), ~21,700 (sh, 2600), ~23,500 (sh, 3200), 27,200 (12,400), 30,100 (11,100), 34,000 (23,100), 34,700 (sh, 22,100)
Ni(MeHMe-2,9-diene)[c,g]	dia	14,500 (195), 15,800 (175), ~17,300 (sh, 120), 21,100 (3620), 22,700 (3560), 27,500 (18,500), 28,700 (17,000), 33,100 (20,300), 36,800 (22,300)
H_2(Et$_2$(ch)oaa)[c]		31,100 (24,100), 39,500 (30,400)
Ni(Et$_2$(ch)oaa)[c]	dia	17,080 (3770), ~17,900 (sh, 3380), ~22,200 (sh, 5200), 22,700 (5730), 30,800 (12,300), ~37,900 (sh, 11,900)
Cu(MeHMe(en)$_2$)[c,g]	1.78	16,200 (111), 20,900 (sh, 220), 22,500 (sh, 330), 24,700 (sh, 790), 27,700 (sh, 5900), 29,500 (21,800), 30,200 (sh, 13,000), 33,200 (10,300), ~40,000 (sh, ~12,500)
[Cu(MeHMe-2,9-diene)](BF$_4$)[f]	1.11	12,740 (1890), ~14,820 (sh, 2190), 15,400 (2230), ~17,600 (sh, 1300), 21,600 (1110), ~23,100 (sh, 1380), ~27,500 (sh, 13,400), 29,200 (21,900), 32,100 (19,000), 33,800 (21,400), ~34,800 (sh, 19,500), 37,000 (17,600)
Cu(MeHMe-2,9-diene)[c,g]	1.78	15,100 (170), 16,600 (160), 21,000 (3240), 22,200 (3810), ~23,500 (sh, 2750), ~26,000 (sh, 5850), 28,700 (32,700), ~29,500 (sh, 29,600), ~33,500 (sh, 11,800), 35,400 (17,300), ~43,000 (sh, 28,000)

[a] Solid state, ~25°; dia = diamagnetic. [b] Apparent values, uncorrected for underlying absorption. [c] Chloroform solution. [d] DMF solution. Due to extreme air sensitivity of the Fe(II) complex, a reproducible spectrum at >10,500 cm^{-1} was not obtained. [e] Methanol solution. [f] Acetonitrile solution. [g] Reproducible spectra were not obtained at <14,000 cm^{-1} because of decomposition in ir lamp beam. [h] $c = 9.5 \times 10^{-4}$ M.

Table IV. Conductivity Data for Acetonitrile Solutions at 25° [a]

Compound	Λ_o ohm^{-1} cm^2 equiv^{-1}	A Calcd	A Found
(n-Bu$_4$N)(BF$_4$)	163	350[b]	362
[Ni(H(MeHMe(en)$_2$))](BF$_4$)	167	352[b]	369
[Ni(MeHMe-2,9-diene)](BF$_4$)	179	748[c]	580
[Cu(MeHMe-2,9-diene)](BF$_4$)	167	352[b]	355

[a] The cell was calibrated with 0.01 N KCl in distilled water. Measurements were made at four–six concentrations in the range 5×10^{-3}–1×10^{-4} M. [b] Calcd for 1:1 electrolyte. [c] Calcd for 2:1 electrolyte. Value for 1:1 electrolyte is 362.

monium tetrahalometalate(II) salts were employed as the anhydrous metal(II) source. Due to the slowness of the reactions at room temperature, they were conducted at 50° for 12–48 hr. At the conclusion of the reactions the solvent was removed under reduced pressure and the product extracted with and recrystallized from dry, degassed xylene under nitrogen atmosphere. The cobalt(II) complex was isolated as an orange-red crystalline solid which is apparently stable to dry air; it is air-sensitive in solution. The iron(II) complex was obtained as a brick-red solid which is sensitive to atmospheric oxygen in both solid and solution phases. Yields of the two purified complexes were *ca.* 50%.

M(MeHMe(en)$_2$), M = Ni(II), Cu(II). Reaction of hot ethanolic solutions containing equimolar proportions of H$_2$(MeHMe(en)$_2$) and the appropriate metal(II) acetate hydrate afforded the desired complexes in crystalline form. The nickel(II) complex was further purified by recrystallization from dry xylene and was isolated as red-brown needles. The copper(II) complex was recrystallized under nitrogen from dry degassed xylene and was obtained as emerald green platelets. Yields of purified products were 80–95%.

Table V. Polarographic Data for Nickel and Copper Complexes

Complex	Solvent	Couple[a]	$E_{1/2}$, V	i_d/C[b] (μA/mM)	Slope, mV $\lvert E_{1/4} - E_{1/4}\rvert$
Ni(MeHMe-2,9-diene)	CH_3CN	$0 \rightleftarrows +1$	+0.11	−29	64
		$+1 \rightleftarrows +2$	+0.70	−24	54
[Ni(MeHMe-2,9-diene)](BF$_4$)	CH_3CN	$0 \rightleftarrows +1$	+0.10	33	59
		$+1 \rightleftarrows +2$	+0.70	−26	52
Cu(MeHMe-2,9-diene)	CH_3CN	$0 \rightleftarrows +1$	−0.04	−25	58
		$+1 \rightleftarrows +2$	+0.50	−26	73
[Cu(MeHMe-2,9-diene)](BF$_4$)	CH_3CN	$0 \rightleftarrows +1$	−0.07	26	60
		$+1 \rightleftarrows +2$	+0.50	−25	57
Ni(MeHMe(en)$_2$)	DMF	$0 \rightleftarrows +1$	+0.11	−14	64
Ni(MeHMe(NH)$_2$)$_2$	DMF	$0 \rightleftarrows +1$	+0.18	−12	52
Cu(MeHMe(en)$_2$)	DMF	$0 \rightleftarrows +1$	+0.17	−15	61
Zn(MeHMe(en)$_2$)	CH_3CN	$0 \rightleftarrows +1$	+0.23	−22	86
Ni(MeHH(phen)$_2$)	DMSO	$-1 \rightleftarrows 0$	−1.63	7.0	61
		$0 \rightleftarrows +2$	+0.58	−17	55
Ni(HHH(phen)$_2$)	DMSO	$-1 \rightleftarrows 0$	−1.66	8.0	64
		$0 \rightleftarrows +2$ (?)	+0.65	−12	74
Ni(Me, COMe, H(phen)$_2$)	CH_3CN	$-1 \rightleftarrows 0$	−1.48	25	62
		$0 \rightleftarrows +1$	+0.94	−24	68
		$+1 \rightleftarrows +2$	+1.14	−24	73
Ni(Me$_2$(ch)oaa)	CH_2Cl_2	$-1 \rightleftarrows 0$	−1.20	25	57
		$0 \rightleftarrows +2$[c]	+1.26	−43	71
Ni(Et$_2$(ch)oaa)	CH_2Cl_2	$-1 \rightleftarrows 0$	−1.24	23	61
		$0 \rightleftarrows +2$[c]	+1.25	−40	65

[a] Notation used indicates number of electrons transferred but does not necessarily imply strict electrochemical reversibility. [b] Comparison data for one-electron transfer: $[Ni(tdt)_2]^{2-} \rightleftarrows [Ni(tdt)_2]^- + e^-$, i_d/C (μA/mM) = 25 (CH_3CN); 12 (DMF); 6.8 (DMSO); 18 (CH_2Cl_2). [c] The total oxidation waves of these complexes appear to be comprised of two overlapping processes. The $E_{1/2}$ and slope values given are for the more anodic component and the i_d/C values are given for the total oxidative diffusion current. In each case, the less positive portion of the wave has a diffusion current of *ca.* $\leq$ 40% of the total and appears to be markedly irreversible.

2,11-Diimonium-4,9-dimethyl-5,8-diazadodeca-3,9-diene Tetrafluoroborate (10). This compound was obtained by isolating the white solid formed during 3 hr of stirring as described in the preceding preparation. This material was collected by filtration under dry nitrogen and recrystallized from *ca.* 800 ml of absolute methanol to yield 18–20 g (31–35%) of pure product. No attempt was made to recover additional product from the filtrate. The product is hydrolytically unstable and should be handled under a dry nitrogen atmosphere.

H$_2$(MeHMe(en)$_2$) from 10 and Ethylenediamine. Sodium (0.46 g, 20 mmol) was dissolved in 100 ml of absolute methanol under a dry nitrogen atmosphere. To this solution was added 3.98 g (10 mmol) of **10** followed immediately by 0.60 g (10 mmol) of ethylenediamine. The reaction solution was stirred for 3 hr at room temperature while ammonia was evolved. Removal of methanol under reduced pressure followed by extraction of the residue with 200 ml of hot absolute ethanol and cooling of the extract solution yielded white crystals. Recrystallization from absolute ethanol afforded 1.3 g (52%) of crystalline product identified by melting point and pmr as H$_2$(MeHMe(en)$_2$).

M(MeHMe(en)$_2$), M = Fe(II), Co(II). These complexes were prepared by the nonaqueous chelation reaction in *tert*-butyl alcohol which has been described in detail previously.[20] Tetraalkylammonium tetrahalometalate(II) salts were employed as the anhydrous metal(II) source. Due to the slowness of the reactions at room temperature, they were conducted at 50° for 12–48 hr. At the conclusion of the reactions the solvent was removed under reduced pressure and the product extracted with and recrystallized from dry, degassed xylene under nitrogen atmosphere. The cobalt(II) complex was isolated as an orange-red crystalline solid which is apparently stable to dry air; it is air-sensitive in solution. The iron(II) complex was obtained as a brick-red solid which is sensitive to atmospheric oxygen in both solid and solution phases. Yields of the two purified complexes were *ca.* 50%.

Zn(MeHMe(en)$_2$). This complex was synthesized by nonaqueous chelation[21,22] by utilizing n-butyllithium in tetrahydrofuran (distilled under a nitrogen atmosphere from lithium aluminum hydride). To a solution of 6 mmol of H$_2$(MeHMe(en)$_2$) in 200 ml of THF maintained at −20° was added 2 equiv of a 1.6 M n-butyllithium solution in n-hexane under a dry nitrogen atmosphere. Tetraethylammonium tetrachlorozincate(II) (6 mmol) was added and the reaction mixture stirred at room temperature for 6 days. Removal of the solvent under reduced pressure followed by extraction of the product with and recrystallization from dry degassed xylene yielded golden brown platelets in 65% yield. The product was dried *in vacuo* for 12 hr before analysis. The compound is sensitive to moisture and should be stored under a dry nitrogen atmosphere.

Dihydro-1,2,4,5,8,9,11,12-octaaza[14]annulenes, H$_2$(R$_2$(ch)$_2$oaa) and Their Nickel(II) Complexes, Ni(R$_2$(ch)$_2$oaa) (26). H$_2$(R$_2$(ch)$_2$-oaa), R = Me, Et. Both of these compounds have been reported recently[17] but preparative details were not given. The methyl derivative was obtained by the following method. 1,2-Cyclo-

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hexanedione dihydrazone (2.5 g, 18 mmol) dissolved in 100 ml of absolute ethanol was added to a solution of 2.9 g (18 mmol) of triethyl orthoacetate in 50 ml of absolute ethanol containing 1 drop of concentrated sulfuric acid. The mixture was heated at *ca.* 60° for 24 hr under nitrogen. Red crystals appeared after 7 hr. The reaction mixture was cooled and the red crystalline product collected by filtration. After drying for 1 hr *in vacuo* 0.6 g (21%) of product was obtained. The ethyl derivative was prepared by an analogous procedure employing triethyl orthopropionate. A red crystalline product was obtained in 16% yield. The two compounds were identified by their melting points and pmr spectra.[17]

Ni(R_2(ch)$_2$oaa), R = Me, Et. To a solution of 1.5 mmol of the free base in 175 ml of hot absolute ethanol was added 0.38 g (1.5 mmol) of nickel acetate tetrahydrate in 50 ml of hot absolute ethanol. The violet solid which separated was collected and air-dried. The complexes were purified by recrystallization from 1:1 v/v toluene–*n*-heptane and were isolated in 74–78% yield as violet crystals.

[Ni(H(MeHMe(en)$_2$))](BF$_4$) (12). In attempts to prepare complexes derived from the neutral macrocycle **11**, reactions of it and nickel(II) salts were carried out under several different conditions. Salts of the 1:1 type were obtained; the following procedure was the most reliable. H_2(MeHMe(en)$_2$) (3.0 g, 12 mmol) was dissolved in 100 ml of degassed absolute ethanol under a nitrogen atmosphere and 7.8 g (12 mmol) of tetraethylammonium tetrabromonickelate(II) in 100 ml of degassed absolute ethanol added. The reaction mixture was refluxed for 24 hr and filtered when hot in the absence of air. To the warm filtrate was added 2.7 g of sodium tetrafluoroborate in 50 ml of hot degassed methanol. Upon cooling red crystals separated. This material was purified by repeated (3–4 times) recrystallization from a degassed 1:1 v/v mixture of absolute methanol–ethanol under a nitrogen atmosphere. The product was obtained as deep red platelets (1.5 g, 31%), which should be protected from the atmosphere.

Reaction Products of Ni(MeHMe(en)$_2$). **(a) [Ni(MeHMe-2,9-diene)](BF$_4$) (15, M = Ni).** The following operations were carried out under a nitrogen atmosphere. To a solution of 4.0 g (13 mmol) of Ni(MeHMe(en)$_2$) in 30 ml of dry degassed acetonitrile was added 13.6 g (39 mmol + 5% excess) of trityl tetrafluoroborate.[23] The solution was heated at ~50° for 5 hr. During this time the solution became dark green and then dark green crystals separated. After cooling overnight a quantity of green-black crystals and a small amount of yellowish white crystals (triphenylmethane) were collected by filtration. The latter were removed by washing with degassed absolute ethanol. Purification was accomplished by two recrystallizations from ~450 ml of 1:1 v/v mixture of dry degassed acetonitrile–ethanol. The yield of green-black needles was 2.5 g (48%). The compound should be protected from the atmosphere.

(b) Ni(MeHMe-2,9-diene) (14, M = Ni). The following operations were carried out under a nitrogen atmosphere. [Ni(MeHMe-2,9-diene)](BF$_4$) (0.50 g, 1.3 mmol) was suspended in 25 ml of degassed absolute ethanol and 49 mg (1.3 mmol) of sodium borohydride was added. The mixture was stirred at room temperature for 4 hr. During the first 20 min, rapid gas evolution and a change in solution color from green to golden brown occurred. The microcrystalline solid which separated was collected and dried *in vacuo*. The pure complex was obtained by two recrystallizations from *ca.* 300 ml of dry degassed xylene (green solution) and isolated as copper-colored platelets, which were dried *in vacuo* overnight yielding 0.33 g (85%) of product.

Reaction Products of Cu(MeHMe(en)$_2$). **(a) [Cu(MeHMe-2,9-diene)] (BF$_4$) (15, M = Cu).** The following operations were carried out under a nitrogen atmosphere. To a solution of 4.0 g (13 mmol) of Cu(MeHMe(en)$_2$) in 30 ml of dry degassed acetonitrile was added 13.6 g (39 mmol + 5% excess) of trityl tetrafluoroborate.[23] The mixture was stirred at ~50° for 8 hr. It turned dark red-brown almost immediately and after *ca.* 30 min, a dark green color developed and dark green crystals began to separate. At the end of the reaction period the mixture was cooled and filtered to yield large green-black crystals, which were washed with ~50 ml of absolute ethanol and twice recrystallized from 500 ml of a 1:1 v/v mixture of dry degassed acetonitrile–ethanol. Additional crops were isolated in both recrystallizations by reducing the volume of the filtrate. A total of 1.7–2.1 g (34–41%) of green-black crystals was obtained. The product should be protected from the atmosphere.

(b) Cu(MeHMe-2,9-diene) (14, M = Cu). The following operations were carried out under a nitrogen atmosphere. [Cu(MeHMe-2,9-diene)](BF$_4$) (0.40 g, 1.0 mmol) was suspended in 30 ml of degassed absolute ethanol and 39 mg (1.0 mmol) of sodium borohydride added. The mixture was stirred at room temperature for 6 hr. During the first 30 min, vigorous gas evolution took place and the crystals of the starting material were converted to a brown microcrystalline solid. The mixture was filtered and the brown solid collected was dried *in vacuo*. Three recrystallizations from ~300 ml of dry degassed xylene afforded 0.24 g (78%) of pure product as brown platelets, which were dried *in vacuo* at 80° for 3 days. The product should be protected from the atmosphere.

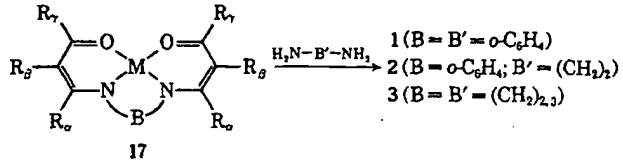
Physical Measurements. Electronic spectral data were obtained using a Cary Model 14 spectrophotometer. Magnetic measurements were made by the Faraday method using HgCo(NCS)$_4$ and Ni(en)$_3$S$_2$O$_3$ as calibrants. A Mechrolab Model 302 osmometer operating at 37° was used for molecular weight measurements in solutions prepared from dry toluene. A Princeton Applied Research Model 170 electrochemistry system was employed for electrochemical measurements. The usual polarographic measurements were carried out using a rotating platinum electrode as the working electrode. Solutions were ~10^{-3} *M* in complex and 0.10 *M* in tetra-*n*-butylammonium perchlorate as the supporting electrolyte in dichloromethane solution and 0.05 *M* tetra-*n*-butylammonium tetrafluoroborate as the supporting electrolyte in other solvents. All potentials were determined at 25° *vs.* a saturated calomel reference electrode. Pmr spectra were obtained on either a Varian HR-100 or a Hitachi Perkin-Elmer R-20B spectrometer using TMS as an internal standard. Conductivity measurements were made using as the solvent acetonitrile which had been distilled from calcium hydride under a nitrogen atmosphere and a Serfass conductivity bridge. Solutions were thermostated at 25.0 ± 0.2° during the measurements.

Mass Spectra. Low-resolution mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6D spectrometer operating at 70 eV. High-resolution mass spectra were determined using a CEC21-11B double-focusing spectrometer employing photoplate recording and operating at 70 eV. Tabulated below are the principal peaks in the parent ion region of the high-resolution spectra of Ni(MeHMe-2,9-diene) and Cu(MeHMe-2,9-diene). Given in the tabulation are the assigned isotopes and the observed and calculated exact masses for each. The following approximate masses together with the observed (calculated) relative intensities were obtained under low resolution in the parent ion region of Ni(MeHMe-2,9-diene): 300, 100 (100); 301, 19 (17); 302, 43 (40); 303, 9 (8); 304, 8 (6); 306, 2 (2).

Ni(MeHMe-2,9-diene)		
Ion$^+$	Obsd	Calcd
$^{12}C_{14}{}^{1}H_{18}{}^{14}N_4{}^{58}Ni$	300.0857	300.0884
$^{12}C_{13}{}^{13}C_1{}^{1}H_{18}{}^{14}N_4{}^{58}Ni$	301.0901	301.0918
$^{12}C_{14}{}^{1}H_{18}{}^{14}N_4{}^{60}Ni$	302.0828	302.0863
$^{12}C_{14}{}^{1}H_{18}{}^{14}N_4{}^{61}Ni$	303.0851	303.0841
$^{12}C_{14}{}^{1}H_{18}{}^{14}N_4{}^{62}Ni$	304.0791	304.0814
$^{12}C_{14}{}^{1}H_{18}{}^{14}N_4{}^{64}Ni$	306.0799	306.0811
Cu(MeHMe-2,9-diene)		
Ion$^+$	Obsd	Calcd
$^{12}C_{14}{}^{1}H_{18}{}^{14}N_4{}^{63}Cu$	305.0806	305.0829
$^{12}C_{13}{}^{13}C_1{}^{1}H_{18}{}^{14}N_4{}^{63}Cu$	306.0832	306.0863
$^{12}C_{14}{}^{1}H_{18}{}^{14}N_4{}^{65}Cu$	307.0826	307.0809
$^{12}C_{13}{}^{13}C_1{}^{1}H_{18}{}^{14}N_4{}^{65}Cu$	308.0855	308.0843

Results and Discussion

Synthesis of Macrocycle and Complexes. Jager[3,6,9,10] has demonstrated that macrocyclic complexes 1–3 can be prepared by reactions between the tetradentate β-ketoamine complexes **17** (M = Ni, Cu) and primary diamines. The occurrence or extent of reaction appears to be significantly dependent upon the nature of R_β. Cyclization reactions involving aliphatic diamines



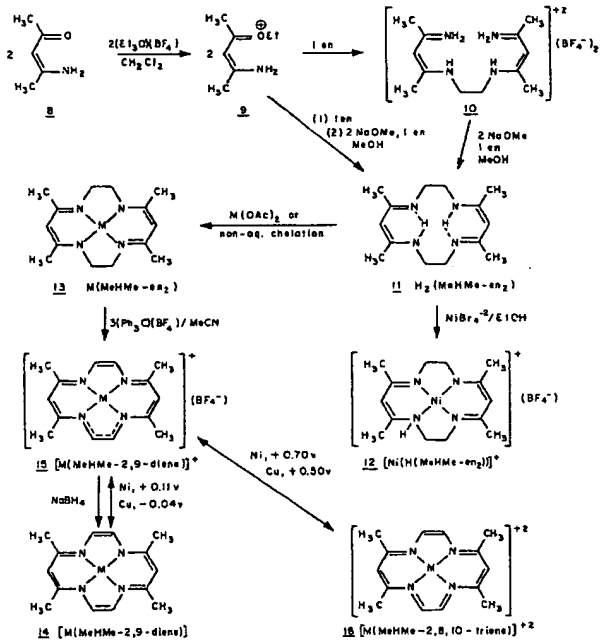
17

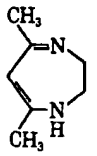
(23) H. J. Dauben, Jr., L. R. Honnen, and K. M. Harmon, *J. Org. Chem.*, 25, 1442 (1960).

fail unless R_β = COR or COOR. For example, we have found that refluxing bis(acetylacetone)ethylenediimine–metal(II) complexes in neat ethylenediamine for up to 2 days did not result in cyclization. In related work Bamfield[11] has found that ring closure can be effected with analogs of **17** derived from 2-hydroxymethylenecyclohexa-1,3-dione but not from 2-hydroxymethylenecyclohexanone. Closure can be accomplished by reaction of *o*-phenylenediamine and **17** with or without carbonyl-containing groups at the β positions.[6] In the latter case strongly forcing conditions are required. We have confirmed the synthesis[6] of Ni(MeHH(phen)$_2$) using molten *o*-phenylenediamine as the reaction medium.

In view of the failure of the above template reactions to yield macrocyclic complexes lacking functional groups, a nontemplate method of synthesis was developed. The reactions employed are set out in Scheme I. Preparation of the macrocycle 5,7,12,14-tetramethyl-1,4,8,11-tetraazacyclotetradeca-4,6,11,13-tetraene (H_2(MeHMe(en)$_2$), **11**) was first accomplished by a reaction sequence in which intermediates were not isolated. Alkylation of the β-ketoamine **8** yielded the *O*-ethyl cation **9**, a dichloromethane solution of which was treated with 1 equiv of ethyenediamine and subsequently with 2 equiv of sodium methoxide and a second equivalent of diamine. Straightforward work-up afforded the pure macrocycle in 35% average yield. Molecular weight determination by osmometry in toluene solution and by mass spectrometry provides the necessary demonstration that the product is not the monomer 2,3-dihydro-5,7-dimethyl-1,4-diazepine[24] which has structure **18** in weakly polar media.[25] Ultraviolet and pmr spectral data (*cf.* Tables II and III) are consistent with those reported for noncyclic β-iminoamines[25b,26–28] and thus support structure **11**. In particular the strongly deshielded resonance of the NH proton (−11.6 ppm) is indicative of the existence of hydrogen-bonded chelate rings. The course of the

Scheme I. Synthesis of the Macrocycle **11** and Its Derivative Ni and Cu Complexes.





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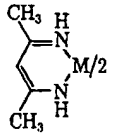
reaction has been investigated by isolating the product formed from **9** and the first equivalent of ethylenediamine. The 1:2 salt **10** was obtained in 31–35% yield. The exact tautomeric structure of the cation is uncertain. Reaction of the salt with 2 equiv of base and 1 equiv of diamine gave the macrocycle **11** in 52% yield. These results indicate that the macrocycle is formed by two nucleophilic reactions. The first involves attack of the amine nucleophile at the–COEt carbon of **9** and is analogous to similar reactions of alkylated β-ketoamine cations with amines and hydrosulfide ion to afford β-iminoamines[28] and β-aminothiones.[22] The second reaction is an analogous nucleophilic attack on the neutral base obtained from **10**, resulting in displacement of ammonia by the stronger base ethylenediamine and concomitant ring closure. No attempt has been made to isolate any products other than **11** which may have been formed in this reaction.

The neutral 12-π complexes M(MeHMe(en)$_2$) (**13**) are readily obtained from the macrocycle by reaction with the metal(II) acetate (Ni(II), Cu(II)) or by nonaqueous chelation (Fe(II), Co(II), Zn(II)). As a class they are moderately soluble in weakly polar solvents. The ligand structure requires an essentially planar M–N_4 geometry, although this stereochemistry is apparently preferred on an electronic basis inasmuch as McGeachin's sterically unencumbered bis(β-iminoaminato) complexes **19** (M(MeHMe–(NH)$_2$)$_2$), M = Co(II), Ni(II), Cu(II), are planar in the solid and solution phases.[28] Magnetic and ligand field spectral



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data for the M(MeHMe(en)$_2$) complexes of these metal ions (Table III) are closely comparable with those of types **2** and **3**,[9] **7**[15] (B = CH_2CH_2), and **19**.[28] All Co(II) complexes are low spin and exhibit a d–d band near 11,000 cm^{-1}. The lowest energy ligand field transition occurs at 16,200 and 17,900 cm^{-1} for the spin doublet Cu(II) and diamagnetic Ni(II) complexes, respectively. Fe(MeHMe(en)$_2$) is of particular interest for it is one of the few well-characterized, apparently four-coordinate ferrous complexes. Its magnetic moment of 3.95 BM in the solid phase is consistent with an S = 1 ground state and finds analogy with the value (3.89 BM) at ambient temperature for Fe(II) phthalo-

(24) G. Schwarzenbach and K. Lutz, *Helv. Chim. Acta*, 23, 1139 (1940); D. Lloyd and D. R. Marshall, *J. Chem. Soc.*, 2597 (1956).

(25) (a) H. A. Staab and F. Vögtle, *Chem. Ber.*, 98, 2701 (1965); (b) E. Daltrozzo and K. Feldman, *Ber. Bunsenges. Phys. Chem.*, 72, 1140 (1968).

(26) J. E. Parks and R. H. Holm, *Inorg. Chem.*, 7, 1408 (1968).

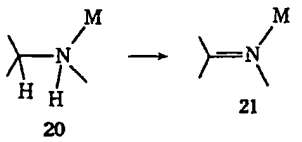
(27) C. L. Honeybourne and G. A. Webb, *Spectrochim. Acta, Part A*, 25, 1075 (1969).

(28) S. G. McGeachin, *Can. J. Chem.*, 46, 1903 (1968).

cyanine, whose electronic structure has been deduced to be $(xz,yz)^4(xy)^1(z^2)^1$ from magnetic anisotropy measurements.[29] Related measurements have not as yet been performed on Fe(MeHMe(en)$_2$) and its electronic ground state remains to be established. Both this complex and Co(MeHMe(en)$_2$) are markedly oxygen sensitive in solution and this behavior is currently under investigation.

Transformations of M(MeHMe(en)$_2$). One of our principal interests in macrocyclic tetraaza complexes of the 6-6-6-6, 6-6-6-5, and 6-5-6-5 types containing one or more saturated rings is concerned with their transformation into species whose conjugated ligand structures are related to those of natural macrocycles such as porphyrins and corrins. Although the 6-5-6-5 complexes **13** do not reproduce the ring-size patterns found in the biological ligands, they serve as feasible starting points for development of the desired reactions. These complexes lack the possibly interfering functional groups R_β = COR, COOR present in **3**.[3,6,9,10] Double dehydrogenations of each of their dimethylene bridges result in a fully conjugated and, hence, presumably stable 16-π ligand system. Such 16-π complexes, which have been obtained previously only as the di-*o*-phenylene bridged species **1**, have in addition the potentiality of generating a three-membered electron transfer series whose terminal oxidized member could have a 14-π ($4n + 2$) stabilized ligand structure.

The systematic transformation of a parent macrocyclic tetraaza complex into species with greater or lesser degrees of unsaturation was first achieved by Curtis.[12a] By dehydrogenation and hydrogenation reactions he was able to transform the *cis*- and *trans*-tetraazadiene complexes **5** into their tetraazatetraene (**4**) and fully saturated analogs, respectively. This work,[12a] together with other investigations,[33-37a] has demonstrated that coordinated secondary amines[37b] may be oxidatively dehydrogenated to imines (**20** → **21**). A similar approach was attempted in this work.



(1)

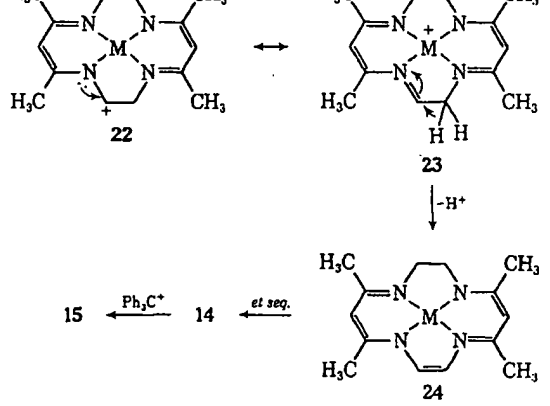
20 **21**

Reaction of the macrocycle **11** with Ni(II) in the absence of base afforded, instead of the anticipated dipositive complex cation, the monoprotonated species **12** (Scheme I). Analytical results, nmr data (Table II), and conductivity in acetonitrile (Table IV) are consistent with the indicated formulation. Attempts to effect the conversion **20** → **21** with several different oxidizing agents were not successful.

Attention was then directed to reactions involving loss of hydrogen, either as H^+ or H^- or both, from the five-membered rings of the complexes **13**, which do not contain coordinated secondary amines. The ultimately successful reactions were suggested by the work of Bonthrone and Reid,[38] who found that additional conjugation could be introduced into certain unsaturated hydrocarbons by the reaction sequence 2 and 3. Hydride abstraction by the strongly electrophilic trityl cation affords a resonance-stabilized carbonium ion which undergoes proton elimination resulting in the formation of a carbon–carbon double bond. As indicated in Scheme I, reaction of M(MeHMe(en)$_2$), M = Ni(II) and Cu(II), with 3 equiv of trityl tetrafluoroborate in acetonitrile effects dehydrogenation of the five-membered chelate rings. The overall process may be interpreted in terms of reactions 2 and 3, in which case the cation **22** → **23** is initially formed and then eliminates a proton resulting in the species **24**. Repetition of the sequence would generate the fully conjugated complex **14**, which is oxidized to the cation **15** by the third equivalent of trityl cation.[39] The cationic species **15**, [M(MeHMe-2,9-diene)]$^+$, were isolated in the form of their crystalline tetrafluoroborate salts. Upon treatment with sodium borohydride in ethanol, the cations were smoothly reduced to the corresponding neutral complexes **14**, M(MeHMe-2,9-diene), which were obtained in yields of *ca.* 80% after purification. The conversion of M(MeHMe(en)$_2$) (12-π) to M(MeHMe-2,9-diene) (16-π) is one of the few examples of

$$-\text{CHRCHR}'- + \text{Ph}_3\text{C}^+ \longrightarrow [-\text{CHRCR}'-]^+ + \text{Ph}_3\text{CH} \quad (2)$$

$$[-\text{CHRCR}'-]^+ \longrightarrow -\text{RC}{=}\text{CR}'- + \text{H}^+ \quad (3)$$



(29) C. G. Barraclough, R. L. Martin, S. Mitra, and R. C. Sherwood, *J. Chem. Phys.*, 53, 1643 (1970). There are few magnetic data reported for Fe(II)–N_4 chelates. Moments of Fe(II) tetraphenylporphin have been claimed to be 0 BM[30] (benzonitrile solution) and 4.75 BM[31] (solid) implying S = 0 and 2 in the two different phases. The only other Fe(II) complex with a moment similar to that of Fe(MeHMe(en)$_2$) is bis(monothioacetylacetone)ethylenediimine–Fe(II)[32] (3.80 BM).

(30) A. Wolberg and J. Manassen, *J. Amer. Chem. Soc.*, 92, 2982 (1970).

(31) H. Kobayashi, M. Shimizu, and I. Fujita, *Bull. Chem. Soc. Jap.*, 43, 2335 (1970).

(32) D. M. C. Wei and S. C. Cummings, Abstracts of Papers, 162nd National Meeting of the American Chemical Society, Sept 1971, INOR-136.

(33) V. L. Goedken, Abstracts of Papers, 162nd National Meeting of the American Chemical Society, Sept 1971, INOR-150.

(34) E. Ochiai and D. H. Busch, *Inorg. Chem.*, 8, 1798 (1969).

(35) E. K. Barefield and D. H. Busch, *ibid.*, 10, 108 (1971).

(36) E. G. Vassian and R. K. Murmann, *ibid.*, 6, 2043 (1967).

(37) (a) J. C. Dabrowiak, F. V. Lovecchio, V. L. Goedken, and D. H. Busch, Abstracts of Papers, 162nd National Meeting of the American Chemical Society, Sept 1971, INOR-140. (b) Recently the oxidative dehydrogenation of a coordinated primary amine has been reported: B. C. Lane, J. E. Lester, and F. Basolo, *Chem. Commun.*, 1618 (1971).

(38) W. Bonthrone and D. H. Reid, *J. Chem. Soc.*, 2773 (1959).

(39) The individual steps 13 → (22, 23) → 24 → 14 are suggested by analogy to reactions 2 and 3;[38] studies of separate reaction steps have not been carried out. In particular, the proposed intermediate **24** has not been isolated. It is noted that the initial complexes **13**, like **14**, possess rather low polarographic half-wave potenials (Table V) and their oxidation by trityl cation could be the first step in the reaction sequence instead of hydride abstraction as shown. Reaction of **13** with 2 equiv of trityl tetrafluoroborate also afforded the cation **15** in *ca.* 30% yield. If the above sequence is correct, this result indicates that under the preparative conditions employed, the effective rate of oxidation of **14** is comparable to or exceeds the rate of hydride abstraction from **13** by trityl cation.

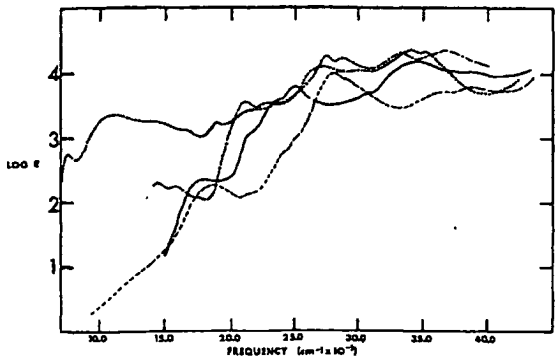


Figure 1. Electronic spectra of nickel complexes: (——) Ni(MeHMe(en)$_2$) (13) in chloroform; (– – – –) [Ni(H(MeHMe(en)$_2$))](BF$_4$) (12) in methanol; (····) Ni(MeHMe-2,9-diene) (14) in chloroform; (· · · ·) [Ni(MeHMe-2,9-diene)](BF$_4$) (15) in acetonitrile (9.5×10^{-4} *M*). Spectra of 13 and 14 could not be obtained below *ca.* 14,000 cm^{-1} due to photolytic decomposition.

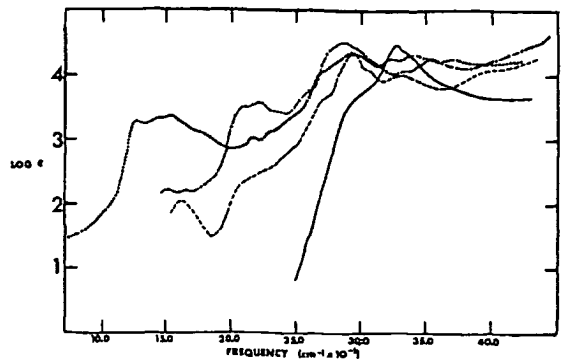
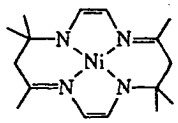


Figure 2. Electronic spectra of the macrocycle 11 and copper complexes: (——) H$_2$(MeHMe(en)$_2$) (11) in chloroform; (– – – –) Cu(MeHMe(en)$_2$) (13) in chloroform; (– · – ·) Cu(MeHMe-2,9-diene) (14) in chloroform; (· · · ·) [Cu(MeHMe-2,9-diene)](BF$_4$) in acetonitrile. Spectra of 13 and 14 could not be obtained below *ca.* 14,000 cm^{-1} due to photolytic decomposition.

introduction of carbon–carbon unsaturation in chelate rings and is the only instance of formation of a fully conjugated ligand system by this means. Of the two previous examples,[1b,36] the transformation of the dipositive *trans*-Ni(II) tetraene **4** under basic conditions to the corresponding neutral complex,[1b] formulated here as the 12-π species **25**, is the more closely related to the present work.



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Properties of M(MeHMe-2,9-diene) and [M(MeHMe-2,9-diene)]$^+$. Magnetic and electronic spectral results are given in Table III and Figures 1 and 2. The neutral macrocyclic complexes M(MeHMe-2,9-diene) were isolated as brown crystalline solids, which are only slightly soluble in weakly polar solvents yielding green solutions. Low solubility precluded determination of solution molecular weights and measurement of the pmr spectrum of the diamagnetic nickel complex. Confirmatory evidence for the formulation of these complexes as the fully conjugated 16-π species **14** has been obtained from their high-resolution mass spectra (*cf.* Experimental Section). The complexes are monomeric in the vapor phase and in each case the most intense spectral peaks are those of the molecular ions ($^{12}C_{14}{}^1H_{18}{}^{14}N_4{}^{58}Ni$ and $^{12}C_{14}{}^1H_{18}{}^{14}N_4{}^{63}Cu$). The nickel complex was also examined under low-resolution conditions and the observed and calculated relative intensities of isotope peaks in the molecular ion region were found to be in satisfactory agreement. The electrochemical studies described below reveal that the nickel and copper complexes **14** and **15** may be interconverted by reversible one-electron redox reactions, thereby demonstrating that these species differ only in total oxidation level. Electronic spectral comparisons of the complexes **13** and **14** of each metal reveal a number of differences, the most significant of which is the greater absorbance of the fully conjugated species in the near-infrared region.

The cationic complexes [M(MeHMe-2,9-diene)]$^+$, **15**, were obtained as green-black tetrafluoroborate salts, which are moderately soluble in polar solvents such as acetonitrile, DMF, and DMSO, affording intense green solutions. Their electronic spectra are markedly different from the neutral species **14**. Pronounced absorbance was found in the near-infrared, and no photochemical instability was detected. The origin of the magnetic moment of the solid copper complex (1.11 BM) is unknown at present and could result from intermolecular antiferromagnetic spin coupling or distribution over intramolecular spin singlet and triplet states. The most nearly analogous known complex, Cu(tetraphenylporphine)$^+$, has a moment of 2.88 BM in solution,[30] indicating a triplet spin state. Solubility limitations prevented accurate magnetic measurements in solution. [Ni(MeHMe-2,9-diene)](BF$_4$) was found to be diamagnetic in the solid state at room temperature. Because the cation is an odd-electron species, this result implies spin pairing possibly through dimer or polymer formation in the solid state. The nature of this complex in solution was investigated by the conductivity and spectral measurements; solution magnetic studies could not be performed because of solubility restrictions.

The degree of association n of an electrolyte A_nB_n may be determined from measurement of the equivalent conductivity (Λ_e) as a function of concentration.[40] The slope A obtained from a plot of the Onsager limiting law $\Lambda_0 - \Lambda_e = A\sqrt{c}$ is characteristic of a particular n value in a given solvent. Conductivity data obtained from measurement of acetonitrile solutions (5×10^{-3}–1×10^{-4} *M*) of three complexes prepared in this work and (n-Bu$_4$N)(BF$_4$) as a standard 1:1 electrolyte are given in Table IV. A comparison of calculated and observed A values[41] reveals that tetra-

(40) R. D. Feltham and R. G. Hayter, *J. Chem. Soc.*, 4587 (1964).

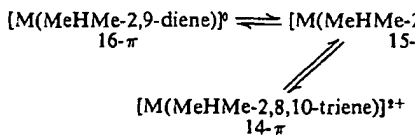
(41) Values of Λ_0 were determined by extrapolation of plots of Λ_e *vs.* $\sqrt{c}$. The slopes A were obtained from these plots. Theoretical values of A were calculated for the electrolyte types (A$^+$)(B$^-$) (1:1) and (A$_2^{2+}$)(B$^-$)$_2$ (2:1) from the limiting law expression using constants appropriate to acetonitrile.[42] In the calculation of A for the 2:1 case the equivalent ionic conductance λ_0^- of tetrafluoroborate in acetonitrile was taken as 108.5 ohm^{-1} cm^2 equiv^{-1} [43] and λ_0^+ was determined by difference, $\Lambda_0 = \lambda_0^- + \lambda_0^+$. Solubility limitations prevented measurement of solutions of the complexes **15** with concentrations greater than *ca.* 5×10^{-3} *M*.

(42) P. Walden and E. J. Birr, *Z. Phys. Chem.*, 144, 269 (1929); J. F. Coetzee and G. P. Cunningham, *J. Amer. Chem. Soc.*, 87, 2529 (1965).

(43) H. L. Yeager and B. Kratochvil, *J. Phys. Chem.*, 73, 1963 (1969).

fluoroborate salts of the nickel complex **12** and the copper complex **15** behave as simple 1:1 electrolytes. The observed value of A for [Ni(MeHMe-2,9-diene)]-(BF_4) is, however, intermediate between the values calculated for 1:1 and 2:1 electrolytes found in this and other[44] conductivity studies in acetonitrile. Spectral studies of this compound in acetonitrile over the same concentration interval used in the conductivity measurements reveal definite departure from Beer's law and isosbestic points at 18,500 and 22,000 cm^{-1}. No spectral changes were observed at concentrations above *ca.* 2.0×10^{-3} M. Similar spectral behavior is encountered in 3:1 v/v DMF–acetone solutions, whose epr spectra reveal a signal of $\langle g \rangle = 2.002$, assigned to the spin-doublet monomer, with no resolved hyperfine splitting (peak-to-peak width 5.5 G). The spectrum of a frozen solution (*ca.* −100°) consists of a single signal with no detectable anisotropy. From these preliminary results it is considered likely that [Ni(MeHMe-2,9-diene)]$^+$ exists in a paramagnetic monomer ⇌ diamagnetic dimer equilibrium. Preliminary analysis indicates that the spectrum shown in Figure 1 corresponds to *ca.* <10% monomer present.

Polarographic Results. (a) [M(MeHMe-2,9-diene)]^z Complexes. Synthesis of the fully conjugated complexes **14** immediately raised the possibility that they would be subject to two one-electron oxidations, producing a heretofore unknown three-membered electron transfer series terminating in species with a 14-π ($4n + 2$)-stabilized ligand system. The polarographic data in Table V and the cyclic voltammograms shown in Figure 3 indicate that the nickel and copper complexes each undergo two well-separated reversible or quasi-reversible one-electron oxidations in acetonitrile solution. Further, the cations **15** exhibit both a one-electron oxidation and a one-electron reduction at half-wave potentials within 30 mV of the corresponding processes of the neutral complexes.[45] Slopes of the polarographic waves of the cations correspond very closely to the theoretical value of 56 mV for a reversible one-electron transfer. No other waves were observed in the potential interval of *ca.* −2.0 to +2.0 V. These results lead to the formulation of the following three-membered electron transfer series (*cf.* Scheme I) in which the redox processes are proposed to effect



changes in total oxidation levels of the macrocyclic ligands with the metals retaining a formal M(II) oxidation state. The epr data for the nickel cation described above are consistent with a π-radical description. The dipositive cations **16** have not yet been isolated. In terms of previous considerations[46] the series is considered "complete" in that its limits are defined by the terminal reduced and oxidized forms of the ligand system in combination with a stable valence state of the metals employed. It is limited to three members rather than the five found for the similarly complete series $[M(C_6H_4(NH)_2)_2]^z$ [46] and $[Pt(R_2NCS-NNPh)_2]^z$ [47] ($z = -2$ to $+2$), whose greater redox capacity arises from combinations of different oxidation states of separate ligands coordinated to the same metal.

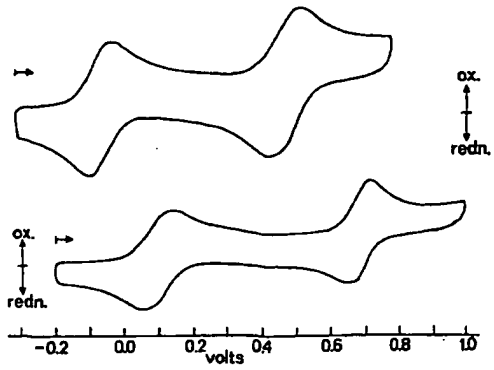


Figure 3. Cyclic voltammograms of the complexes **14** in acetonitrile: upper, Cu(MeHMe-2,9-diene); lower, Ni(MeHMe-2,9-diene). The voltammograms were recorded at a sweep rate of 0.50 V/sec using a stationary platinum electrode.

The redox behavior of the complexes **14**, **15**, and **16** contrasts with that of Ni(II) and Cu(II) complexes of types **5** and **6**. The latter usually exhibit single-electron oxidation and reduction processes,[48] but these are characterized by potentials which are more anodic and much more cathodic than those which interrelate members of the above series. In these cases the ligands lack independent redox capacity and the processes may be reasonably interpreted as M(II) → M(I) and M(II) → M(III).[48]

(b) Related 16-π Systems. In an attempt to assess the generality of the 16-π ⇌ 14-π electron transfer series, the polarographic behavior of two other systems related to the fully conjugated complexes **14** have been investigated. The octaaza[14]annulene complexes **26**[49] and the di-*o*-phenylene bridged complexes **1** potentially can be oxidized to the M(II) 14-π dications **27** and **28**, respectively. Polarographic results for a

(44) A. L. Balch, I. G. Dance, and R. H. Holm, *J. Amer. Chem. Soc.*, 90, 1139 (1968); A. Davison, D. V. Howe, and E. T. Shawl, *Inorg. Chem.*, 6, 458 (1967).

(45) Cyclic voltammetric studies of [Ni(MeHMe-2,9-diene)](BF_4) in acetonitrile have established the reversibility of the oxidation and reduction processes of the cation. Polarographic wave slopes are then not consistent with a reversible two-electron transfer per dimeric unit. Diffusion currents given in Table V for this complex were calculated using monomeric formula weights. This procedure accords with the conductivity and spectrophotometric data if a rapid monomer ⇌ dimer equilibrium obtains and only the monomer is electroactive in the potential range employed. In other solvents and with different supporting electrolytes some evidence has been obtained for redox processes involving one-electron per dimeric unit. These processes are currently under investigation.

(46) A. L. Balch and R. H. Holm, *J. Amer. Chem. Soc.*, 88, 5201 (1966).

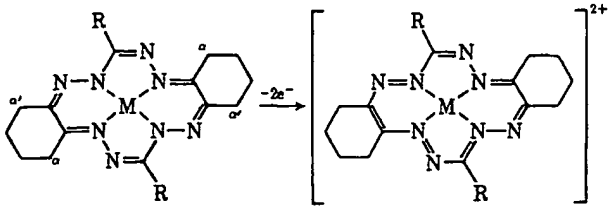
(47) C. E. Forbes, A. Gold, and R. H. Holm, *Inorg. Chem.*, 10, 2479 (1971).

(48) (a) D. C. Olson and J. Vasilevskis, *Ibid.*, 8, 1611 (1969); (b) D. C. Olson and J. Vasilevskis, *ibid.*, 10, 463 (1971); (c) D. P. Rillema, J. F. Endicott, and E. Papaconstantinou, *ibid.*, 10, 1739 (1971). For related results of Cu(II) 6-6-5-5 tetraaza macrocycles, *cf.* L. F. Lindoy, N. E. Tokel, L. B. Anderson, and D. H. Busch, *J. Coord. Chem.*, 1, 7 (1971).

(49) In the initial report of the synthesis of metal-free octaazaannulenes,[27a] a structure was proposed which, upon deprotonation, would afford neutral metal(II) complexes of D_{2h} symmetry. Pmr studies of a variety of these macrocycles and their Ni(II) complexes have established a C_{2h} structure,[50] as shown in **26**. The inequivalence of the α- and α'-methylene groups required by this structure but absent in the D_{2h} case is revealed by the pmr data in Table II for several free bases and their complexes. More recently the C_{2h} structure has been recognized,[17b] but no supporting evidence for it was presented.

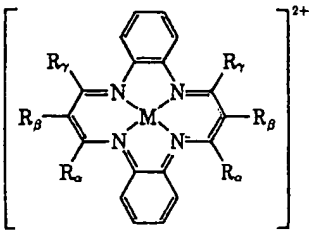
(50) J. E. Baldwin, R. H. Holm, R. E. Harper, J. Huff, S. Koch, and T. J. Truex, *Inorg. Nucl. Chem. Lett.*, 8, 393 (1972).

number of Ni(II) complexes are given in Table V.



26 27

Anodic processes are observed in each case. With



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the exception of Ni(Me, COMe, H(phen)$_2$) these processes correspond to apparent two-electron oxidations, most of which are at best quasi-reversible as judged by wave slopes. The results do imply that the oxidations **26** → **27** and **1** → **28**, the possibility of which was recognized earlier by Jager,[6] can be effected. No chemical oxidations of **1** and **26** have been attempted. Unlike **14** each of these complexes undergoes a one-electron reduction at potentials somewhat less negative than those required for the Ni(II) → Ni(I) reduction of species **5** and **6**.

(c) M(MeHMe(en)$_2$) Complexes. For the purpose of comparison with the fully conjugated complexes **14**, the polarographic behavior of M(MeHMe(en)$_2$), M = Ni(II), Cu(II), and Zn(II), has been investigated. Results are given in Table V. These complexes do not reduce at potentials down to *ca.* −2.0 V but do undergo apparent one-electron oxidations at slightly positive potentials. No other anodic waves were observed. Due to the quite low potentials of these processes and their near-independence of metal ion, they are associated with oxidation of the ligand π system rather than with M(II) → M(III).[48] This interpretation is supported by the occurrence of similar slightly anodic oxidations with Ni(MeHMe(NH)$_2$)$_2$ (**19**, Table V) and [Ni(MeHMe(NCH$_2$CH$_2$NHMe)$_2$)]$^+$,[48a] which also contain delocalized β-iminoaminato chelate ring systems. The close correspondence of half-wave potentials between the oxidation of M(MeHMe(en)$_2$) and the first anodic process of M(MeHMe-2,9-diene) may indicate that they are related. In view of this, it should be emphasized that the representation **15** (Scheme I) for [M(MeHMe-2,9-diene)]$^+$ is only one of a number of simple formulations depicting the delocalized nature of the 15-π ligand system.

Further examples of the nontemplate synthesis of tetraaza macrocyles and additional results dealing with the preparation, reactivity, solution behavior, and electronic properties of complexes of the types **14**, **15**, and **16** will be reported subsequently.

Acknowledgment. This research was supported by grants from the National Institutes of Health (GM-15471) and the National Science Foundation (GP-18978X). The high-resolution mass spectra were provided by the facility supported by National Institutes of Health Grant RR00317 (Professor K. Biemann, Principal Investigator) from the Biotechnology Resources Branch, Division of Research Resources. Useful discussions with Professor A. Davison are acknowledged.

Phosphonitrilic Compounds. XIV.[1] Basic Hydrolysis of Aryloxy- and Spiroarylenedioxycyclophosphazenes[2]

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Contribution from the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802. *Received July 28, 1971*

Abstract: Bis(aryloxy)cyclophosphazenes, [NP(OAr)$_2$]$_3$, and spirocyclic arylenedioxycyclophosphazenes, (NPO$_2$Ar)$_3$, have been hydrolyzed in basic 25 vol % water in diglyme. For the bis(aryloxy) derivatives, the ease of hydrolytic removal of the first aryloxy group in [NP(OR)$_2$]$_3$ from phosphorus is in the order OR = *p*-NO$_2$C$_6$H$_4$O− > *m*-NO$_2$C$_6$H$_4$O− > *o*-NO$_2$C$_6$H$_4$O− ≫ C$_6$H$_5$O− > *p*-CH$_3$C$_6$H$_4$O−. For the spirocyclic derivatives, the rate of cleavage of the first aryloxy–phosphorus bond is in the order [NP(O$_2$C$_6$H$_4$-1,2)]$_3$ > [NP(O$_2$C$_{10}$H$_6$-2,3)]$_3$ ≫ [NP(O$_2$C$_{12}$H$_8$-2,2′)]$_3$ and [NP(O$_2$C$_{12}$H$_6$-1,8)]$_3$. The mechanisms of these reactions are discussed, and comparisons are made with related phosphate ester hydrolyses.

Aryloxycyclo- and polyphosphazenes, [NP(OAr)$_2$]$_n$, and spirocyclic phosphazenes, [NP(O$_2$Ar)]$_3$ or [NP(O$_2$Ar)]$_4$, occupy an important place in phosphorus–nitrogen chemistry. Aryloxycyclophosphazenes, such as [NP(OAr)$_2$]$_3$ or [NP(OAr)$_2$]$_4$, are among the most thermally and oxidatively stable phosphorus

(1) Part XIII: H. R. Allcock, R. L. Kugel, and E. G. Stroh, *Inorg. Chem.*, 11, 1120 (1972).

(2) Preliminary report of parts of this work have appeared: H. R. Allcock and E. J. Walsh, *J. Amer. Chem. Soc.*, 91, 3102 (1969); *Chem. Commun.*, 580 (1970).

FULL PAPER

Protonation and coordination properties towards Zn(II), Cd(II) and Hg(II) of a phenanthroline-containing macrocycle with an ethylamino pendant arm

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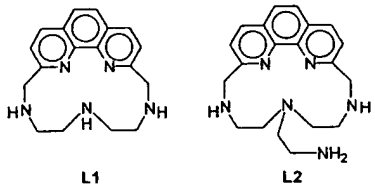
Protonation and Zn(II), Cd(II) and Hg(II) coordination with the ligand 5-aminoethyl-2,5,8-triaza-[9]-10,23-phenanthrolinophane (**L2**), which contains an aminoethyl pendant attached to a phenanthroline-containing macrocycle, have been investigated by means of potentiometric, ^{1}H NMR and spectrofluorimetric titrations in aqueous solutions. The coordination properties of **L2** are compared with those of the ligand 2,5,8-triaza-[9]-10,23-phenanthrolinophane (**L1**). Ligand protonation occurs on the aliphatic amine groups and does not involve directly the heteroaromatic nitrogens. The fluorescence emission properties of **L2** are controlled by the protonation state of the benzylic nitrogens: when not protonated, their lone pairs are available for an electron transfer process to the excited phenanthroline, quenching the emission. As a consequence, the ligand is emissive only in the highly charged $[H_3L2]^{3+}$ and $[H_4L2]^{4+}$ species, where the benzylic nitrogens are protonated. Considering metal complexation, both $[ML1]^{2+}$ and $[ML2]^{2+}$ complexes (M = Zn(II) and Cd(II)) are not emissive, since the benzylic nitrogens are weakly involved in metal coordination, and, once again, they are available for quenching the fluorescence emission. Protonation of the **L2** complexes to give $[MHL2]^{3+}$ species, instead, leads to a recovery of the fluorescence emission. Complex protonation, in fact, occurs on the ethylamino group and gives a marked change of the coordination sphere of the metals, with a stronger involvement in metal coordination of the benzylic nitrogens; consequently, their lone pairs are not available for the process of emission quenching.

There is a continuing interest in the chemistry of polyaza-macrocycles because of their ability to form metal chelates in aqueous solutions and act as selective complexing agents for metal cations. Structural factors, such as ligand rigidity, electron-donor properties of the nitrogens and their disposition, have been shown to play significant roles in determining the binding features of macrocycles toward metal cations.[1–13] Heteroaromatic subunits, such as 2,2′-dipyridine or 1,10-phenanthroline, are often introduced as integral parts of the host molecules.[14–21] These units are rigid and provide two aromatic nitrogens whose unshared electron pairs act co-operatively in binding cations. At the same time, incorporation of these moieties into macrocyclic structures allows to combine within the same ligand the special complexation features of macrocycles with the photophysical and photochemical properties displayed by the metal complexes of these heterocycles.[18] In this respect, these ligands are potential photochemical chemosensors for metals; they are able, in principle, to bind metal cations, and at the same time, to signal its presence in aqueous solution, due to energy- or photoinduced-electron transfer (PET) processes leading to enhancement of the fluorescence emission (CHEF effect) or enhancement of the emission quenching (CHEQ effect) of the heteroaromatic units upon metal coordination.

Earlier we reported on Zn(II) coordination to a series of phenanthroline-containing macrocycles composed by a polyamine chain connecting the 2,9-positions of a phenanthroline unit, such as the ligand 2,5,8-triaza-[9]-10,23-phenanthrolinophane (**L1** in Scheme 1).[19c,e] These ligands are able to give stable Zn(II) complexes in aqueous solution. Although the Zn(II) complexes with phenanthroline-based ligands generally display a marked CHEF effect upon Zn(II) binding, the Zn(II) complexes with these polyamine macrocycles are surprisingly not emissive: this effect was attributed to the benzylic amine groups, whose lone pairs are weakly involved in metal coordination and, therefore, available for an electron transfer process. This leads to a consequent quenching of the fluorescence emission of phenanthroline.[19c,e]



Scheme 1

We have now extended this study to the ligand 5-aminoethyl-2,5,8-triaza-[9]-10,23-phenanthrolinophane (**L2**), which displays an ethylamino group as a pendant arm attached to the macrocyclic framework of **L1**. It has been shown, in fact, that the attachment of an aminoalkyl side arm can strongly affect the coordination properties of the ligand[22–34] as well as the photophysical properties of its complexes.[13,35,36]

To further elucidate the role of metal cations in the fluorescence emissions properties of the complexes, we have also investigated the coordination properties of **L1** and **L2** toward the larger and softer Cd(II) and Hg(II) cations.

Results and discussion

Ligand protonation

The protonation equilibria of **L2** have been studied by means of potentiometric measurements in aqueous solutions and the

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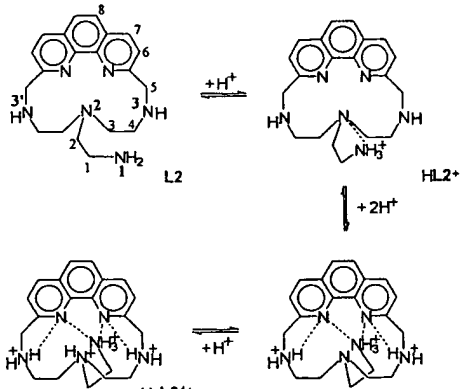
Table 1 Protonation constants of ligands L1 and L2 (NMe_4Cl 0.1 M, 298 K)

Equilibrium	log K L1[a]	log K L2
$L + H^+ = LH^+$	9.99	10.20(2)[b]
$LH^+ + H^+ = LH_2^{2+}$	7.72	8.78(4)
$LH_2^{2+} + H^+ = LH_3^{3+}$	4.11	7.89(6)
$LH_3^{3+} + H^+ = LH_4^{4+}$		2.57(6)

[a] From ref. 19c. [b] This work; values in parenthesis are standard deviations on the last significant figure.

L2 basicity constants are reported in Table 1. The protonation constants of L1, previously determined,[19c] are also reported for comparison. In the case of L1, it was found that all three protonation steps involve the aliphatic amine groups, while the phenanthroline unit remains unprotonated, even in the $[H_3L1]^{3+}$ species, due to the by far lower basicity of phenanthroline nitrogens than aliphatic amine ones. A similar behaviour is also expected for L2. The data in Table 1 clearly show that the first three protonation constants are at least two log units higher than the protonation constant of 1,10-phenanthroline ($\log K = 4.96$),[37] suggesting that these protonation steps occur on aliphatic amine groups. The low value of the fourth protonation constant does not allow, however, to infer hypothesis on the localisation of the fourth acidic proton. The protonation pattern of L2 has been clarified by recording ^1H NMR spectra at different pH values. The ^1H spectrum of L2 at pH 11.7, where the free amine predominates in solution displays five signals for the aliphatic protons and three for the aromatic ones, accounting for a C_{2v} time-averaged symmetry of the ligand, which is preserved over all the pH range investigated (2.5–11.7). The pH dependence of the ^1H NMR signals is reported in Fig. 1, together with the distribution diagram of the protonated species of L2. In the pH range 11.7–9.5, where the first proton binds to the ligand, the most significant changes in the ^1H spectra are the marked downfield shift of the signal of H1, in α-position with respect to N1; this strongly suggests that the first protonation step occurs on the NH_2 group of the pendant arm (Scheme 2).



Scheme 2

The minor downfield shift observed for the protons H2 and H3, adjacent to N2, may suggest the presence of a hydrogen bond between the protonated N1 nitrogen and N2, and/or to a partial localisation of the acidic proton on the N2 tertiary nitrogen. Binding of the second and third proton to give the $[H_3L2]^{3+}$ species occurs in the pH range 9.5–6 and produces a dramatic downfield shift of the signals of H4 and H5, in α-position with respect to the N3 and N3′ benzylic nitrogens, indicating that these protonation steps involve the benzylic

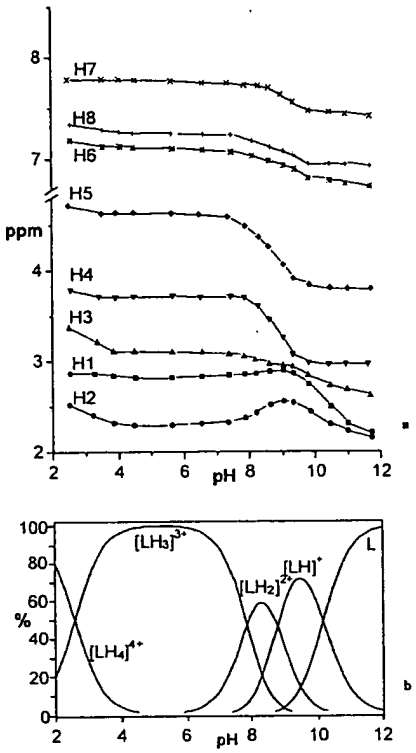


Fig. 1 pH dependence of the ^1H NMR signals of L2 (a) and distribution diagram of the L2 protonated forms (b) ([L2] = 1×10^{-3} M, $I = 0.1$ M).

nitrogens. At the same time, the resonance of H2 shifts upfield. These spectral features indicate that in the triprotonated form of the ligand the acidic protons are localised on the N1, N3 and N3′ nitrogens, while N2 is not involved in proton binding. Such a disposition of the protons in the $[H_3L2]^{3+}$ species would mean a minimum in electrostatic repulsion, since the protons occupy alternate positions, separated from each other by the unprotonated tertiary nitrogen N2 or by the phenanthroline unit. Actually, the downfield shifts of the resonances of protons H2 and H3 below pH 4 indicate that the fourth protonation step takes place on N2, adjacent to two already protonated amine groups, thus accounting for the low value of the fourth protonation constant. Interestingly, Fig. 1 also shows a downfield shift of the signals of the phenanthroline protons with the formation of the di- and triprotonated species of the ligand. Although these shifts are too small to be attributed to protonation of phenanthroline, this would indicate that the protonated aliphatic amine groups give hydrogen bond interactions with the heteroaromatic nitrogens. This hypothesis is confirmed by the analysis of the absorption spectra recorded on solutions containing L2 at various pH values. A slight red shift (5 nm) of the phenanthroline band at 273 nm, in fact, is observed from alkaline to strongly acidic pH values. Significant red shifts upon protonation have been reported,[38] instead, in the case of systems containing the chromophore 1,10-phenanthroline but not bearing amine groups. As shown in Fig. 2, a slight progressive increase of the absorbance at 268 nm is observed with the formation of the di-, tri- and tetraprotonated forms of the ligand, due to the formation of hydrogen bonds involving the phenanthroline nitrogens upon ligand protonation.

In contrast with absorption, the fluorescence emission intensity is very dependent on the protonation state of L2. A total quenching of the emission, in fact, is observed for the species L2, $[HL2]^+$ and $[H_2L2]^{2+}$, while $[H_3L2]^{3+}$ and $[H_4L2]^{4+}$ exhibit an intense emission (Fig. 2). As already observed in the case of ligand L1, the quenching effect in the less protonated species

Table 2 Selected bond lengths (Å) and angles (°) for the metal coordination environment in the $[HgL1Br]^+$ cation

Hg–N(1)	2.399(8)	Hg–N(4)	2.353(9)
Hg–N(2)	2.386(8)	Hg–N(5)	2.533(8)
Hg–N(3)	2.562(9)	Hg–Br	2.580(2)
N(1)–Hg–N(2)	69.2(3)	N(2)–Hg–Br	120.1(2)
N(1)–Hg–N(3)	135.3(3)	N(3)–Hg–N(4)	73.2(3)
N(1)–Hg–N(4)	111.9(3)	N(3)–Hg–N(5)	145.9(3)
N(1)–Hg–N(5)	68.0(3)	N(3)–Hg–Br	92.0(2)
N(1)–Hg–Br	121.0(2)	N(4)–Hg–N(5)	74.2(3)
N(2)–Hg–N(3)	68.0(3)	N(4)–Hg–Br	115.0(3)
N(2)–Hg–N(4)	111.8(3)	N(5)–Hg–Br	92.4(2)
N(2)–Hg–N(5)	135.5(3)		

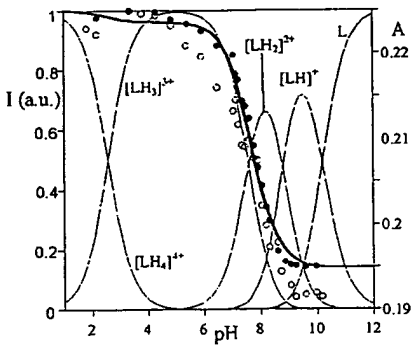


Fig. 2 pH Dependence of the absorbance at 268 nm (○, right y axis) and of the fluorescence emission intensity at 380 nm (●, left y axis, λ_{exc} = 290 nm) of L2 as a function of pH ([L2] = 1.5×10^{-5} M, 0.1 M NMe_4Cl, 298 K), superimposed to the distribution diagram of the protonated species of the ligand.

can be explained by an electron transfer process from the unprotonated amine groups closest to the excited phenanthroline, the benzylic nitrogens N3 and N3'.[19c] As actually shown by the NMR study, in $[H_3L2]^{3+}$ and $[H_4L2]^{4+}$ both benzylic nitrogens are protonated. Their lone pairs are not available for quenching processes and, therefore, these highly protonated species are emissive.

Metal coordination

Crystal structure of [HgL1Br]ClO_4. The crystal structure consists of $[HgL1Br]^+$ complex cations (Fig. 3) and perchlorate anions. Selected bond angles and distances for the metal coordination environment are listed in Table 2. The Hg^{2+} ion is coordinated to the five donor atoms of the macrocycle and by an exogenous bromide anion. The coordination geometry can be best described as a strongly distorted octahedron, where the two heteroaromatic nitrogens (N(1) and N(2)) and the benzylic ones define the equatorial plane (maximum deviation 0.011(9) Å for N(1) and N(2)), while N(4) and the bromide anion occupy the apical positions. All the bond angles, however, strongly deviate from the theoretical values (Table 2). The metal ion lies 0.4133(6) Å above the equatorial plane, shifted toward the bromide ion.

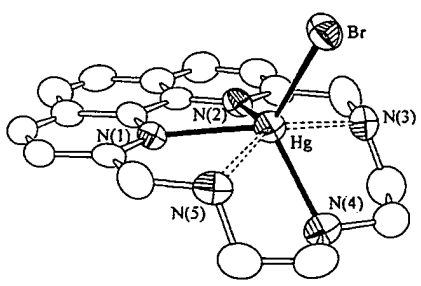


Fig. 3 ORTEP drawing of the $[HgL1Br]^+$ cation

Table 3 Stability constants of the Zn(II), Cd(II) and Hg(II) complexes with L1 and L2 (NMe_4Cl 0.1 M, 298 K)

Equilibrium	log K L1	L2
$L + Zn^{2+} = ZnL^{2+}$	16.15[a]	17.9(1)[b]
$ZnL^{2+} + H^+ = ZnLH^{3+}$		6.21(2)
$ZnL^{2+} + OH^- = ZnL(OH)^+$	4.44	
$ZnL(OH)^+ + OH^- = ZnL(OH)_2$	2.75	
$L + Cd^{2+} = CdL^{2+}$	17.20(5)[b]	18.83(2)[b]
$CdL^{2+} + H^+ = CdLH^{3+}$		6.99(5)
$CdL^{2+} + OH^- = CdL(OH)^+$	2.72(6)	
$L + Hg^{2+} = HgL^{2+}$		30.28(2)[b]
$Hg^{2+} + L + Cl^- = HgClL^+$	28.7(2)[c]	
$HgL^{2+} + H^+ + Cl^- = HgClLH^{2+}$		7.79(7)
$HgClLH^{2+} + H^+ + Cl^- = HgCl_2LH_2^{2+}$		4.49(9)

[a] From ref. 19c. [b] This work, from potentiometric measurements. Values in parenthesis are standard deviations on the last significant figure. [c] From spectrofluorimetric measurements.

The data in Table 2, however, show that the two benzylic nitrogens N(3) and N(5) are bound at a larger distance than the two heteroaromatic donors and the central nitrogen N(4) of the aliphatic chain. In this respect, the phenanthroline nitrogens N(1) and N(2), N(4) and the bromide anion display a rather regular tetrahedral disposition around the Hg(II) ion. A similar disposition of the donors was also found in the crystal structure of the Zn(II) complex $[ZnL1(H_2O)]^{2+}$.[19c] In this complex, the metal is coordinated to the phenanthroline nitrogens, the central amine groups of the aliphatic chain and an exogenous water molecule in a resulting tetrahedral geometry, while the benzylic nitrogens are located *ca.* 2.5 Å apart from the metal. As in $[ZnL1(H_2O)]^{2+}$, the overall conformation of the macrocyclic ligand in $[HgL1Br]^+$ is folded along the axis connecting the benzylic nitrogens, with a dihedral angle of 61.0(3)° between the mean planes defined, respectively, by the benzylic nitrogens and the aromatic unit and by the three secondary nitrogens of the aliphatic chains.

The crystal packing of $[HgL1Br]ClO_4$ displays pairs of symmetry related $[HgL1Br]^+$ cations interacting through face-to-face π-stacking between the two phenanthroline units (interplanar distance 3.57(1) Å). These dimeric units are associated *via* face-to-face π-stacking interactions between phenanthroline units belonging to two different pairs of cations (interplanar distance 3.60(1) Å) into pillars growing up along the a axis.

Zn(II), Cd(II) and Hg(II) complexation in aqueous solutions. Zn(II), Cd(II) and Hg(II) complexation with ligands L1 and L2 was studied by means of potentiometric measurements and the stability constants of the complexes formed in aqueous solutions are listed in Table 3. The low solubility of the Hg(II) complexes with L1 does not allow a speciation study by using potentiometric measurements in aqueous solutions. However, the formation constant of the Hg(II) complex could be determined by means of spectrofluorimetric measurements and its value is also reported in Table 3. In this case, a $[HgL1Cl]^+$ complex is formed in our ionic medium (NMe_4Cl), due to the high affinity of Hg(II) for the chloride anion.

Both ligands form stable complexes with the metal under investigation. Complex formation occurs at acidic pH values and, in the case of Zn(II) and Cd(II) complexation with L1, is followed by deprotonation of coordinated water molecules to give hydroxo-complexes at alkaline pH. In the case of Zn(II) complexation with L1, however, it was found that the benzylic nitrogens are only weakly involved in metal coordination.[19c] The rigidity of the phenanthroline unit, in fact, stiffens the macrocyclic structure, precluding the simultaneous participation of all amine nitrogens in Zn(II) coordination. Actually,

the stability constants of the complexes with L1 are generally lower than the corresponding constants with the pentadentate macrocyclic ligand 1,4,7,10,13-pentaazacyclopentadecane (L3), where an ethylenediamine unit replaces the phenanthroline moiety of L1 (for instance, $\log K = 16.15$ for $[CdL1]^{2+}$ *vs.* $\log K = 19.2$ for $[CdL3]^{2+}$).[39] In the case of the $[ML3]^{2+}$ complexes all five donors are involved in metal coordination. On the other hand, 1,10-phenanthroline and the aliphatic amine *N,N'*-dimethylethylenediamine show similar binding ability toward Zn(II) and Cd(II) ($\log K = 6.55$[40] and 5.47[41] for the equilibrium $Zn^{2+} + L = [ZnL]^{2+}$ and $\log K = 4.9$[42] and 5.2[43] for the equilibrium $Cd^{2+} + L = [CdL]^{2+}$ with L = 1,10-phenanthroline and *N,N'*-dimethylethylenediamine, respectively), and, in the case of Hg(II), 1,10-phenanthroline forms much more stable complexes than *N,N'*-dimethylethylenediamine ($\log K = 19.65$ and 11.78 for Hg(II) complexation with 1,10-phenanthroline and *N,N'*-dimethylethylenediamine, respectively).[43] These considerations suggest that the lower stability of the L1 complexes cannot be simply ascribed to the different binding ability of phenanthroline with respect to an ethylenediamine chain, but it is probably due to the stiffened macrocyclic structure of L1, which does not allow all the nitrogen donors to form strong coordination bonds with the metal ions. This suggestion is supported by the crystal structure of the $[HgL1Br]^{+}$ cation, which shows a distorted octahedral coordination environment for the Hg(II) ion, with both the benzylic amine groups coordinated at a longer distance than the remaining nitrogen donors.

These structural characteristics strongly affect the fluorescence emission features of the complexes.

Similarly to Zn(II), Cd(II) coordination usually gives a marked CHEF effect, *i.e.*, an increase of the fluorescence emission upon metal coordination. In principle, coordination of Cd(II) to the amine nitrogens should prevent the electron transfer quenching process, merely by coulombic effects.[44] In the present case, instead, the formation of the $[CdL1]^{2+}$ complex gives rise to a quenching of the fluorescence emission of phenanthroline. The absence of emission of the $[CdL1]^{2+}$ can be related to the weak involvement of the benzylic amine groups in metal coordination. These nitrogens are coplanar and close to the fluorophore, and therefore, in $[CdL1]^{2+}$ the lone pairs of the benzylic nitrogens can participate in an electron transfer process, quenching the fluorescence emission of the Cd(II) complex.

Similarly to Cd(II), the Hg(II) complex is also not emissive. As shown in Fig. 4, the formation of this complex gives complete quenching of the fluorescence emission. In this case, however, the quenching process is due to the "heavy-atom" effect of the Hg(II) cation.[45] The titration curve in Fig. 4 also allows to determine the stability constant of the Hg(II) complex with L1 ($\log K = 28.7$, Table 3)

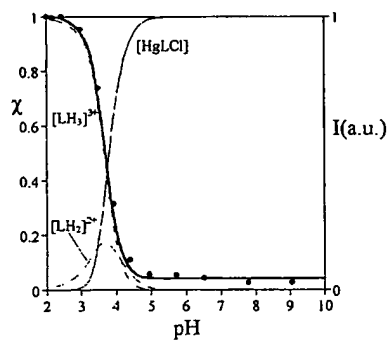


Fig. 4 Fluorescence emission intensity at 380 nm (●, right *y* axis; λ_{exc} = 290 nm) and molar fractions (χ) of the protonated (---) and complexed species of L1 (—, left *y* axis) in the presence of Hg(II) (1 : 1 molar ratio) as a function of pH (λ_{exc} = 290 nm, [Hg(II)] = [L1] = 1.76×10^{-5} M, I = 0.1 M NMe_4Cl, 298 K).

Considering Zn(II), Cd(II) and Hg(II) complexation with L2, the data in Table 3 clearly shows that the insertion of an ethylamino side arm on the L1 cyclic framework leads to an increase of stability of the $[ML]^{2+}$ complexes, accompanied by a marked tendency to form monoprotonated $[ML2H]^{3+}$ complexes. Metal complexation takes place at acidic pH to give $[ML2H]^{3+}$ species, which are prevalent in aqueous solution up to pH 6–7, where deprotonation of the complexes affords the $[ML2]^{2+}$ complexes. In the case of Hg(II) complexation, protonation of the complex is accompanied by chloride binding, with the formation of $[HgCl_xL2H_x]^{2+}$ species. The higher stability displayed by the L2 complexes can be simply related to the involvement of the amino group of the pendant arm in metal coordination, as often observed in macrocyclic complexes bearing alkylamino functionalities.[22–34] Differently from L1, the $[ZnL2]^{2+}$ and $[CdL2]^{2+}$ complexes do not form any hydroxo-complex, indicating that in the coordination sphere of the metal cations is more fulfilled by the donor atoms of the ligand than in the corresponding L1 complexes. At the same time, the fact that the L1 complexes do not display any tendency to form protonated complexes would suggest that protonation of the $[ML2]^{2+}$ species occurs on the primary amine group of the side arm. In the case of Hg(II), detachment of the ethylamino donor implies coordination of a chloride anion to give, in our experimental conditions (0.1 M NMe_4Cl), a $[HgClL2H]^{2+}$ protonated complex, likely due to the high affinity of Hg(II) for the chloride anion. To confirm our hypothesis on proton localisation in the monoprotonated complexes, we decided to perform ^1H NMR titrations on the L2 complexes. Fig. 5 reports the pH dependence of the ^1H NMR signals of the Cd(II) complex with L2, compared with the distribution diagram of the Cd(II) complexes. The most interesting finding is the significant upfield shift of the signals of the ethylenic chain H1 and H2 of the ethylamino pendant arm observed upon protonation of $[CdL2]^{2+}$ to give the monoprotonated $[CdL2H]^{3+}$ species in the pH range 6–8. This strongly suggests that the formation of $[CdL2H]^{3+}$ implies detachment from the metal of the ethylamino group, due to protonation of the N1 primary amine function. In the same pH range, the resonances of the phenanthroline protons do not display any noticeable shift, while the signals of the methylene groups H4 and H5, adjacent to the benzylic nitrogens, display a significant downfield shift. This spectral feature would suggest an increased interaction of the benzylic nitrogens with the metal cation upon detachment of the alkylamino pendant, with the formation of stronger coordinative bonds, as sketched in Fig. 6. Similar changes in the ^1H NMR chemical shifts of aliphatic protons are also observed in the case of protonation of the $[ZnL2]^{2+}$ complex. The hypothesis of an enhanced involvement of the benzylic amine groups in metal coordination is in accord with the fluorescence emission titrations carried out on solutions containing L2 and Zn(II) or Cd(II) in 1 : 1 molar ratio. Fig. 7 shows that the $[ML2]^{2+}$ complexes (M = Zn(II), Cd(II) and Hg(II)) are non-emissive. In the case of the Zn(II) and Cd(II) complexes, the fluorescence emission is recovered upon complex protonation to give the $[ZnL2H]^{3+}$ or $[CdL2H]^{3+}$species. While the lack of emission of the $[ZnL2]^{2+}$ and $[CdL2]^{2+}$ complexes can be simply ascribed to an electron transfer process involving the weakly coordinated benzylic nitrogen, as already observed in the corresponding L1 complexes, the fluorescence emission observed for the monoprotonated species can be related to the enhanced interaction of the benzylic nitrogens in metal coordination, occurring upon protonation and detachment of the ethylamino group. In other words, in $[ZnL2H]^{3+}$ or $[CdL2H]^{3+}$ the lone pairs of the benzylic nitrogens are more strongly involved in metal binding and cannot participate in the electron transfer process responsible for the emission quenching.

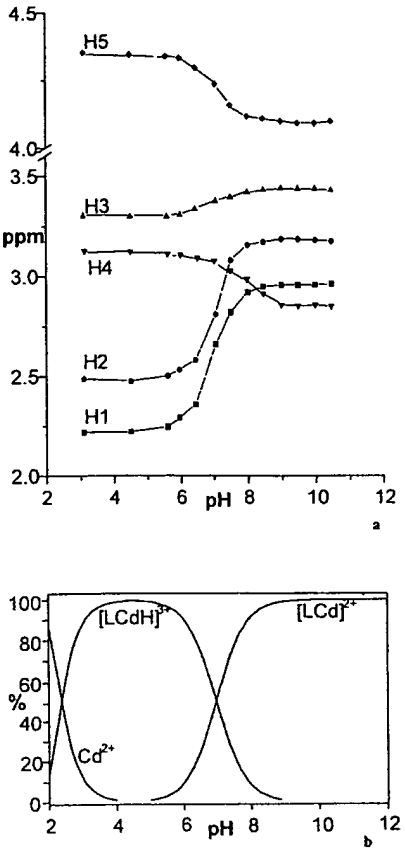


Fig. 5 pH Dependence of the ^{1}H NMR chemical shifts of the L2 complexes with Cd(II) (a) and distribution diagram for the system Cd(II)–L2 (b) ([Cd(II)] = [L2] = 1×10^{-3} M, $I = 0.1$ M). The chemical shifts of the phenanthroline protons do not display significant changes in the pH range investigated and are not reported.

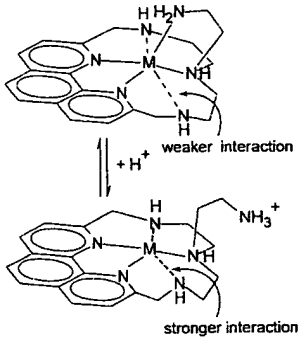


Fig. 6 Sketches of the proposed metal coordination environments in the $[ML2]^{2+}$ and $[ML2H]^{3+}$ complexes.

As already found in the case of L1, the Hg complex with L2 is non-emissive even in its protonated form, due to, once again, the "heavy atom" effect of the Hg(II) cation[45]

The Zn(II) and Cd(II) complexes with L2 are examples of systems whose fluorescence emission is controlled by the protonation state of the ligand, *i.e.*, by the pH of the medium. Although examples of similar pH-controlled fluorescent systems have been reported,[13,35,36,46,47] most of them are constituted by a binding moiety for metals, such as a polyamine macrocycle, and a separated fluorophore pendant arm. In L2, instead, the binding unit and the fluorophore are gathered together within the same macrocyclic framework and the fluorescence emission is determined by the pH-controlled movement of a simple ethylamino side arm.

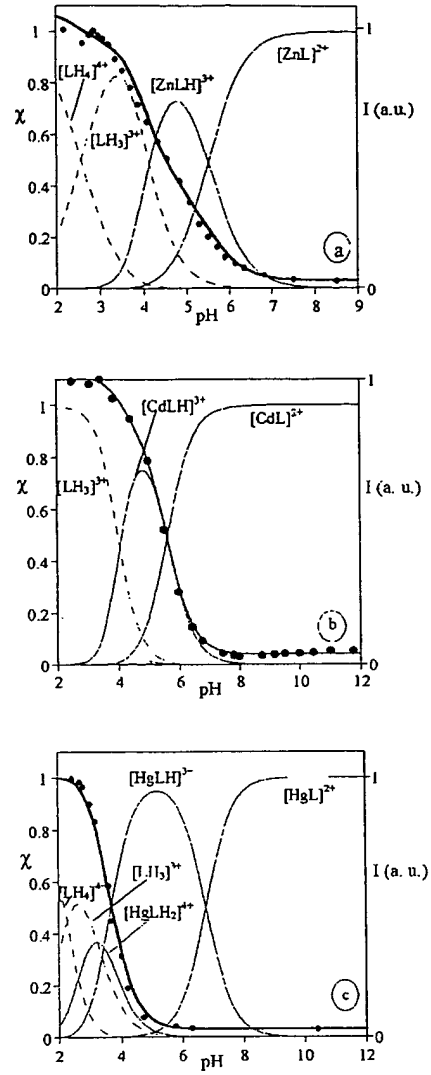


Fig. 7 Fluorescence emission intensity at 380 nm (●, left y axis; λ_{exc} = 290 nm) and molar fractions (χ) of the protonated (---) and complexed species of L2 (—, left y axis) in the presence of Zn (II) (a), Cd(II) (b) and Hg(II) (c) as a function of pH. ([M(II)] = [L2] = 1.5×10^{-5} M, $I = 0.1$ M NMe_4Cl, 298 K).

Experimental

Synthesis

Ligands L1[19c] and L2[20c] were obtained as previously reported. Crystals of $[HgL1Br]ClO_4$ were obtained by slow evaporation at room temperature of an aqueous solution containing ligand L1 and $HgBr_2$ in equimolecular ratio in the presence of an excess of $NaClO_4$.

Potentiometric measurements

Equilibrium constants for L2 protonation and complexation reactions with L1 and L2 were determined by means of potentiometric measurements (pH = $-\log[H^+]$), carried out in 0.1 mol dm^{-3} NMe_4Cl at 298.1 ± 0.1 K, in the pH range 2.5–11, by using the equipment that has been already described.[48] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by Gran's method.[49] This allows one to determine the standard

potential $E°$, and the ionic product of water (pK_w = 13.83 ± 0.01). (1–2) × 10^{-3} mol dm^{-3} ligand and metal ion concentrations were employed in the potentiometric measurements. At least three measurements (about 100 experimental points for each system) were performed for each system. The computer program HYPERQUAD[50] was used to calculate the stability constants of metal complexes from emf data. In the case of Hg(II), the formation of ternary complexes with chloride was taken into account, by using the stability constants for the formation of Hg(II) chloro complexes from ref. 43. For all systems under investigation, the titration curves for each system were treated either as a single set or as separated entities without significant variations in the values of the protonation or metal complexation constants.

X-Ray structure analysis

Formula: $C_{18}H_{21}BrClHgN_5O_4$, monoclinic, space group $P2_1/n$, M = 687.35, a = 8.315(5), b = 23.272(5), c = 11.159 (1) Å, β = 96.53(2)°, Z = 4, V = 2145(1) Å^3, T = 298 K, $F(000)$ = 1312. Data collection: P4 SIEMENS X-ray diffractometer, λ = 1.5418 Å (Cu-Kα), graphite monochromated, 0.15 × 0.1 × 0.1 mm. 3540 Reflections collected (θ_{max} = 59.90°). The structure was solved by direct methods using the SIR-97 program.[51] Refinement was performed by means of the full-matrix least squares method of the SHELX-97 program.[52] All non hydrogen atoms were anisotropically refined. Hydrogen atoms were introduced in calculated positions and their coordinates and thermal factors were refined in agreement with the linked atoms. Refinement included 271 parameters on 2682 unique reflections. Final agreement factors were $R1$ = 0.0396 ($I > 2\sigma(I)$), $wR2$ = 0.1018 (all data).

CCDC reference number 225564.

See http://www.rsc.org/suppdata/dt/b3/b315608g/ for crystallographic data in CIF or other electronic format.

^{1}H NMR measurements

300.07 MHz ^{1}H spectra in D_2O solutions at different pH values were recorded at 298.1 K in a Varian Unity 300 MHz spectrometer. Peak positions are reported relative to HOD at 4.79 ppm. ^{1}H–^{1}H and ^{1}H–^{13}C 2D correlation experiments were performed to assign the signals. In ^{1}H NMR titrations, the pD was adjusted by addition of small amounts of 0.1 mol dm^{-3} NaOD or DCl solutions to solutions containing the ligand alone or in the presence of equimolecular amounts of $Zn(ClO_4)_2·6H_2O$ or $Cd(ClO_4)_2·6H_2O$. The ionic strength was 0.1 M NMe_4Cl. The pH was calculated from the measured pD values using eqn. (1):[53]

$$pH = pD - 0.40 \quad (1)$$

Photophysical studies

All aqueous solutions were prepared in 0.1 mol dm^{-3} NMe_4Cl at 298.1 ± 0.1 K. HCl and NaOH were used to adjust the pH values that were measured on a Metrohm 713 pH meter. Absorption spectra were recorded on a Perkin-Elmer Lambda 6 spectrophotometer and fluorescence emission spectra on a Horiba–Jobin Yvon-Spex Fluorolog 3.22 spectrofluorimeter equipped with a ThermoNeslab RTE7 bath. All the fluorescence spectra were corrected for the instrumental response of the system. The formation constant of the Hg(II) complex with **L1** was determined by least square fitting of the spectrofluorimetric data points derived from the titration carried out on the Hg(II)–**L1** system.

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